A STUDY ON BACTERIOLOGICAL PROFILE OF NECROTISING FASCIITIS IN A TERTIARY CARE HOSPITAL

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APRIL 2016

CERTIFICATE

This is to certify that this dissertation titled "A STUDY ON BACTERIOLOGICAL PROFILE OF NECROTISING FASCIITIS IN A TERTIARY CARE HOSPITAL" is a bonafide record of work done by DR. G.K.ASHA, during the period of her Post Graduate study from 2013 to 2016 under guidance and supervision at the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-600003, in partial fulfillment of the requirement for M.D MICROBIOLOGY degree Examination of The TamilnaduDr. M.G.R Medical University to be held in April 2016.

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DECLARATION

I declare that the dissertation entitled **"A STUDY ON BACTERIOLOGICAL PROFILE OF NECROTISING FASCIITIS IN A TERTIARY CARE HOSPITAL"** submitted by me for the degree of M.D. is the record work carried out by me during the period of September 2013 – August 2014 under the guidance of **Dr.S. Thasneem Banu M.D., Professor, Institute of Microbiology, Madras Medical College,** Chennai. This dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical Unversity, Chennai, in partial fulfilment of the University regulations for the award of degree of M.D., Branch IV (Microbiology) examination to be held in April 2016.

Place: Chennai **Signature of the candidate**

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Introduction:
Necrotising Fasciitis (NF) or Necrotising soft tissue infection (NSTI) is infrequent but is a rapidly progressing lethal infection with necrosis of surrounding tissue.
Necrotising Fasciitis is defined as infection of any of the layers within the soft tissue compartment (dermis, subcutaneous tissue, superficial fascia, deep fascia or muscle) which are associated with necrotising changes. This also includes infections of perineum of both men and women. (1)
NF has a world-wide incidence of 0.001% and carries a mortality rate of 17- 34% (2). It is associated with systemic toxicity and has a fulminant course. Prognosis of NF is dependent on early recognition and treatment. (3)
Some of the predisposing factors are diabetes mellitus, immunocompromised state, usage of corticosteroids , intravenous drug abuse, trauma, malnutrition, burns, and atherosclerosis. (2)
Early diagnosis is the key to manage the disease. NF presents with vague and non-specific symptoms rendering it more complicated to diagnose. Treatment involves a combination of extensive surgical debridement, and antibiotics based on the pathogens. (4)
The prognosis hinges on accurate diagnosis, immediate surgery and initiation of
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CONTENTS

A STUDY ON BACTERIOLOGICAL PROFILE OF NECROTISING FASCIITIS IN A TERTIARY CARE HOSPITAL

ABSTRACT

Necrotising Fasciitis is a rapidly progressing life threatening soft tissue infection with high mortality and morbidity.

OBJECTIVES:

This study is to analyse the frequency of common bacteria causing necrotising fasciitis and antibiotic susceptibility pattern of aerobic isolates. This sudy would also provide an insight into the antimicrobial susceptibility pattern of aerobic isolates.

MATERIALS AND METHODS:

This is a cross sectional study conducted at the Institute of Microbiology in association with the Institute of General Surgery, Madras Medical College, RGGGH, Chennai, during the period of October 2014 to September 2015. One hundred patients diagnosed with Necrotising Fasciitis were included. Tissue samples or wound swabs were collected and processed and identified based on standard microbiological procedures.

RESULTS:

Out of one hundred patientsaffected76% were and 24 % were females. Type II Diabetes mellitus (DM) 59% was the most common co-morbid condition associated with necrotising fasciitis. Type II monomicrobial accounted for 69% and type I monomicrobialconstituted 26% of infections. *Klebsiella pneumoniae*, 29% was the

most common isolate. In Gram positive isolates 10% were *Staphylococcus aureus.*79% were ESBL producers and 42% were MRSA. Anaerobes accounted for 13% of infections in Polymicrobialetiology.

CONCLUSION:

Necrotising fasciitis is a lethal condition wherein high clinical suspicion,prompt diagnosis and extensive surgical and appropriate antibiotic therapy would be necessary to reduce mortality and duration of hospital confinement.

KEY WORDS:

Necrotising Fasciitis, Type II DM,*Klebsiella pneumoniae*, ESBL, MRSA.

INTRODUCTION

Necrotising Fasciitis (NF) or Necrotising soft tissue infection (NSTI) is infrequent but is a rapidly progressing lethal infection with necrosis of surrounding tissue.

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NF has a world-wide incidence of 0.001% and carries a mortality rate of 17- 34% [2]. It is associated with systemic toxicity and has a fulminant course. Prognosis of NF is dependent on early recognition and treatment. [3]

Some of the predisposing factors are diabetes mellitus, immunocompromised state, usage of corticosteroids , intravenous drug abuse, trauma, malnutrition, burns, and atherosclerosis.[2]

Early diagnosis is the key to manage the disease. NF presents with vague and non-specific symptoms rendering it more complicated to diagnose. Treatment involves a combination of extensive surgical debridement, and antibiotics based on the pathogens.[4]

The prognosis hinges on accurate diagnosis, immediate surgery and initiation of appropriate antibiotics and intensive care. [5]

This study is focused on determining the common pathogenic bacteria and their antibiotic susceptibility pattern among the patients of Necrotising Fasciitis. Recognising the common bacterial causes of NF can be of immense assistance in selection of empirical antimicrobial therapy before the results of bacterial cultures are available.

AIMS OF THE STUDY

(1) To determine the common aerobic and anaerobic bacteria causing Necrotising Fasciitis,

(2) To analyse the antibiotic susceptibility of aerobic isolates.

(3) Also to correlate the association of risk factors in patients with necrotising fasciitis.

REVIEW OF LITERATURE

HISTORY

The earliest report of Necrotising Fasciitis dates back to the 5th century BC where Hippocrates describes complication of cellulitis[6]. The first documented case happened in 1194 AD, when Duke Leopold of Austria developed the disease after his foot had been crushed by his horse.

In 1764 Bauriene reported a case of scrotal gangrene which is considered to be the first case published in medical literature.

Joseph Jones, medical officer in confederate army, at the time of American civil war in 1871, studied 2600 cases and described this disease which was common among soldiers due to conditions at the front. The term **"Hospital Gangrene"** was used then. This term Hospital Gangrene was widely in use, because of the condition inside many surgical wards were poor and many of the soldiers contracted the disease during their hospital stay. One other reason was that, they had, trauma of war injuries and amputation was indispensable, which inevitably lead to large wounds which were extremely susceptible to infections. [7].

Jean Alfred Fournier, a French dermatologist in 1883 described scrotal NF in five young males. Fournier considered this condition as idiopathic in his description. Some of the historical descriptions applied to Fournier's gangrene were periurethral, phelgmon, phagedema, or synergistic NF. [8]

In 1924, Frank L. Meleney, an American surgeon depicted the significance of extensive debridement of necrotic tissue to accomplish better results. **"Meleney's Gangrene"** is synonyms with synergistic gangrene that affects the skin and subcutaneous tissue but not deep fascia except in advanced cases where invariably begins as a necrotic ulcer. [9]

In 1952, the term **"Necrotising Fasciitis"** was coined by Dr.B.Wilson, an American surgeon. [10]

Previously several authors advocated that the eponym Fournier to be restricted to cases of idiopathic peripheral gangrene and the term secondary necrotising fasciitis to be used where an established etiology was found. This classification is currently not in use.

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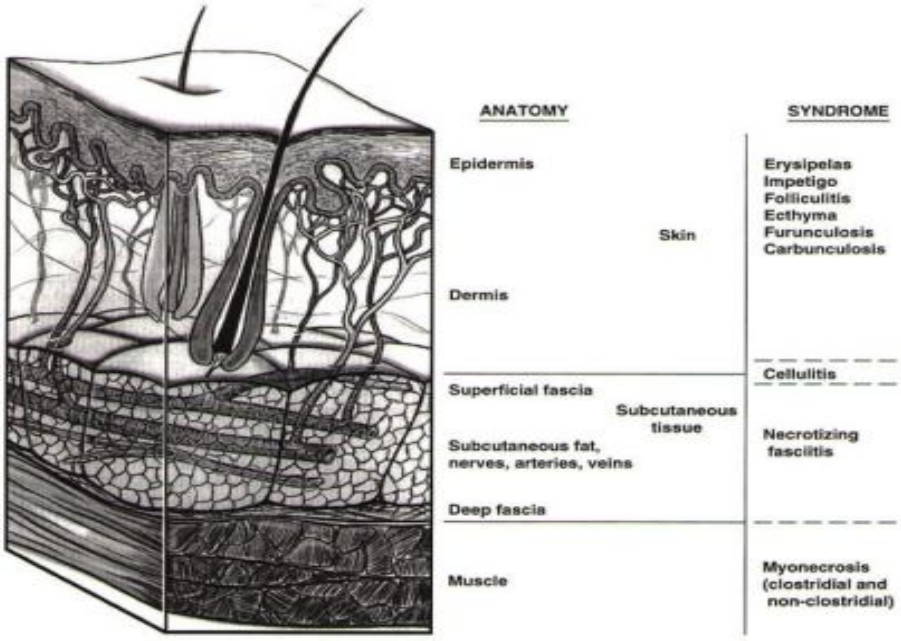


Figure 1

Epidermis of the skin is protected against infection by the mechanical barrier afforded by stratum corneum, since the epidermis itself lacks blood vessels. Erosion or disruption of this layer by bites, abrasion, burns, foreign bodies, primary dermatological disorders (herpes simplex, varicella, and ecthyma gangrenosum) surgery, vascular or pressure ulcers, allows penetration of bacteria to the underlying structures.

Hair follicle also can serve as a portal either for components of normal flora (*staphylococcus*) or for extrinsic bacteria (*Pseudomonas* in hot-tub folliculitis). Bacteria infecting the epidermis are transmitted to the deeper structures through the lymphatics which leads to rapid superficial spread of erysipelas.

The rich plexus of capillaries underneath the dermal papillae serves as a nutritional reservoir to the stratum germinativum, and the physiological responses of this plexus produce important signs and symptoms. The plexus serves as an access for bacteria to the circulation ultimately leading to local spread or bacteraemia.

Exaggeration of any of these physiological processes by overwhelming levels of cytokines or bacterial toxins causes leukostasis, venous occlusion, and pitting oedema. Oedema with purple bullae, ecchymosis, and cutaneous anaesthesia indicating disruption of vascular integrity and requires exploration of the deeper structures for evidence of suggestive of necrotizing fasciitis or myonecrosis. An early diagnosis warrants a high level of clinical suspicion in

instances of unexplained fever, pain and tenderness in the soft tissue, even in the absence of acute cutaneous inflammation.

EPIDEMIOLOGY

The worldwide incidence of Necrotising Fasciitis is estimated to be 0.001%. Some studies has reported 0.4-0.53 cases per 100,000. It is more predominant in males than in the female population. Incidence is higher in patients with diabetes mellitus. Though NF can occur at any site in the body, extremities are more commonly affected followed by perineum and trunk. Mortality rate is reportedly around 17-34 %.[(2)].

PATHOPHYSIOLGY

NF is an infection of the deeper tissues that invariably results in progressive destruction of the muscle fascia and overlying subcutaneous fat. Muscle tissue is frequently spared, due to its generous blood supply. Infection in NF characteristically spreads along the muscle fascia and the overlying tissue which initially appears unaffected. It is this unique feature that makes NF more complicated in diagnosing without surgical intervention. [(12)].

Microbes invade the subcutaneous tissues, either through external trauma or direct spread from a perforated viscus, more commonly from colon or rectum or urogenital organ. Rapid bacterial growth within the superficial fascia results in release of mixture of enzymes endotoxins and exotoxins causing the spread of infection through the fascia. [(13)]

However fibrous attachments which exists between subcutaneous tissues and fasciae confines extension to areas like the scalp, hands, and feet. Deficiency of fibrous attachments in the limbs and trunk, nevertheless leads to extensive infection and tissue destruction. Infection also disseminates to venous circulation and lymphatic channels, resulting in oedema. This dissemination of bacteria results in thrombosis of blood vessels in dermal papilla, leading to ischemic necrosis and gangrene of dermis and subcutaneous fat. Thrombosis of small veins and arteries passing through the fascia causes profound skin ischaemia. [14]

Thus poor microcirculation, ischaemia in affected tissues, ultimately leads to cell death and necrosis. This ischaemia of the skin is the cardinal phenomenon for the necrotising soft tissue infection as it progresses. The skin apparently appears normal in early pathological stages, inspite of extensive infection of the underlying fascia. Haemorrhagic bullae, ulceration, and skin necrosis are the subsequent manifestions with further involvement of the deeper structures. The initial clinical skin findings leads to underestimation of the tissue infection present, even though thrombosis of penetrating vessels to the skin is the distinguishing feature in the pathology of NSTI. Thrombosis of vast numbers of dermal capillary beds must occur before skin changes implicative of necrosis occur. In case of breach in fascia, infection of the muscle occurs leading to myositis. [15]

Vessel occlusion which causes skin infarction and necrosis, facilitates the growth of obligate anaerobes (ex. *Bacteroides*) while contributing to anaerobic metabolism of facultative organisms (ex. *Escherichia coli*), resulting in gangrene. Anaerobic metabolism results in production of gases like hydrogen and nitrogen, which are relatively insoluble gases that accumulates in subcutaneous tissues. [16]

Also organisms that produce gas such as Clostridium species can lead to accumulation of subcutaneous gas, this had resulted in the use of the terminology, gas gangrene. Infections caused by toxin-producing bacteria like *Staphylococcus aureus* and *Streptococcus pyogenes* can lead to toxic shock–like syndrome. Thus, apparently confined infection can result in septic shock and multi-organ failure. [17]. There are two distinct clinical presentations [11]:

(a) Those with no portal of entry and

(b) Those with a defined portal of entry.

Infections in the first category often begin deep at the site of a non-penetrating minor trauma, such as a bruise or a muscle strain. Seeding of the site through transient bacteraemia is likely, however most patients deny antecedent streptococcal infection. These patients present with only severe pain and fever. Later in the course, the classic signs of necrotizing fasciitis, such as purple (violaceous) bullae, skin sloughing and progressive toxicity, develop.

In infections of the second type, *Streptococcus pyogenes* may reach the deep fascia from a site of cutaneous infection or penetrating trauma. These patients

have early signs of superficial skin infection with progression to necrotizing fasciitis.

In either case, toxicity is severe, and renal impairment may precede the development of shock. In 20–40% of cases, myositis occurs concomitantly, and, as in gas gangrene serum creatine phosphokinase levels may be markedly elevated. Gas usually is not present when the cause is *Streptococcus pyogenes* or Methicillin Resistant *Staphylococcus aureus*.

CLASSIFICATION

Current classification of this infection is mainly based on the following:

(a) The plane of tissue affected and the extent of invasion,

(b) The anatomical site,

(c) The causative pathogens.

Deep soft tissue infections are classified either as necrotising fasciitis or necrotising myositis.

Necrotising Fasciitis – rapid, extensive infection of the fascia underneath the adipose tissue.

Necrotising myositis –principally involves the muscles otherwise spreads to adjacent soft tissue. [18]

Classification of soft tissue necrotizing infections[19]

Table:1

Classification	Comments
Anatomic location	Cervical, thoracic, abdominal (Meleney's), pelvis, Fournier's gangrene.
Depth of infection	
(a)Epidermis and dermis	Erysipeals Impetigo Folliculitis Ecthyma Furunculosis Carbunculosis Cellulitis
(b)Superficial fascia, subcutaneous tissue, subcutaneous fat, nerves, arteries, viens, deep fascia.	Necrotising fasciitis
(c)Muscle	Myonecrosis
Microbial causes	Types I, II, III, IV

Classification of soft tissue infections by Lewis. [20]

Infections of skin and subcutaneous tissue

Progressive synergistic bacterial gangrene

Chronic undermining burrowing ulcer (Meleney's ulcer)

Idiopathic scrotal gangrene (Fournier's gangrene)

Infections involving subcutaneous tissue and fascia

Haemolytic *streptococcal* gangrene

Necrotizing fasciitis

Gram-negative synergistic necrotizing cellulitis

Clostridial cellulitis

Infections involving muscle

Clostridial myonecrosis

Streptococcal myositis

Classification based on Microbiology:[21]

Table:2

Types of NF	**Aetiology**	**Organisms**
Type I	Polymicrobial, synergistic, commonly bowel derived	Mixed aerobes and anaerobes
Type II	Monomicrobial, skin or throat derived.	*Group A-β haemolytic Streptococcus*(GAS) and *Staphylococcus aureus*
Type III	Gram negative, marine related organisms	*Vibrio spp*
Type IV (fungal)	Trauma associated	*Candida spp* immunocompromised, zygomycetes – immunocompetent

TYPE I NECROTIZING FASCIITIS

Type I or polymicrobial NF, frequently originates from intestinal flora and synergistic infection. Cultures of tissue will show a mixed growth of anaerobes and aerobes. It is a common type, frequently encountered in patients with diabetes mellitus. Type I NF usually occurs in perineum and trunk of the body. [22]

TYPE II NECROTIZING FASCIITIS

Type II monobacterial, is usually derived from skin or throat infection. The organism causing infection will either be *Group A/B hemolytic Streptococci* alone or in combination with *Staphylococcus aureus*. Type II infections can also be caused by *Staphylococcus aureus* alone.

Type II NF is frequently encountered in young and immuno competent individuals. Type II NF more often occurs due to MRSA. Strains of MRSA that produce the

Panton-Valentine leukocidin (PVL) toxin have been reported to cause necrotizing fasciitis. This infection frequently involves extremities of the body. These patients are prone to toxic shock syndrome and multi organ dysfunction syndrome (MODS). (23, 24)

TYPE III NECROTIZING FASCIITIS

This is the fulminant form of NF, caused by gram negative bacteria, commonly by marine-related organisms. Characteristically occurs after

punctured wound caused by fish or cut injury or an insect bite when exposed to the sea water. It is a hyper acute infection, leads to septic shock and Multi Organ Dysfunction Syndrome within 12 to 24 hours of injury. Early detection is the key to management, and any delay in diagnosis invariably leads to 100% mortality. [25]. A study from Hong Kong showed 83% of their totals NF were type III infection. [26]

TYPE IV NECROTIZING FASCIITIS

Type IV NF occurs in immune compromised and also due severe trauma .Different types Fungi are the major causative agents of infection. It can rapidly spread and results in severe NF. Frequently caused by *candida, Mucor*or *Rhizopus* species. [27]

CLINICAL MANIFESTATION

Most NSTI occurs in the extremities, abdomen, groin, and perineum, however they can occur at any site of the body. Infection begins in the deep tissue planes, hence there might only be a least epidermal involvement. This makes NF complicated, as it has to be distinguished from non-necrotising skin infections and cellulitis. Minor trauma presenting as cellulitis may have deeper plane dissemination and may be missed initially. [28]

Clinical features suggestive of necrotising fasciitis: [29]

Table:3

Skin	**Pain**	**General**
Erythema with ill-defined margins	Pain that extends past margin of apparent infection	Fever with toxic appearance
Tense oedema with or brown discharge	Severe pain, out of proportion to dermal involvement or physical findings	Altered mental state
Lack of lymphangitis or lymphadenopathy	Decreased pain or anaesthesia at apparent site of infection	Tachycardia, tachypnea due to acidosis
Vesicles or bullae, haemorrhagic bullae		Dehydration
Necrosis		Decreased urine output
Crepitus		Presentation with diabetic ketoacidosis.

Erysipelas, being an infection of the superficial dermis, has well-defined borders and often blister. In case of cellulitis, there is predominance of erythema, lymphangitis with minimal blistering.

Necrotizing fasciitis characteristically presents with patchy discolouration of the skin with pain and swelling, but lacks well defined margin and not associated with lymphangitis. [30].As the infection advances, it is more marked with the development of tense oedema, greyish-brown discharge, vesicles, bullae, necrosis, and crepitus. [31]

Haemorrhagic bullae and crepitus are serious signs, indicating extension to underlying fascia and muscle being comprised. [32]. Crepitus being a late sign is only found in 18% of cases of NF. [33]).Usually, there is no pus collection which eventually delays surgical consult or delayed intervention by the surgeon.

Elliot et al and Wang et al in their two retrospective case series reported absence of blisters in 76% to 95% and 62% to 73% respectively, upon initial presentation. [34, 35]

Patients with diabetic neuropathy may have minimal pain leading to a missed diagnosis. This peculiarly occurs in concealed sites of infection, like the perineum or oral cavity. An anaesthetic patch over the site of erythema is also sometimes described in NF. This is presumed to be due to infarction of cutaneous nerves in necrotic subcutaneous fascia and soft tissue. [36]

Some specific types of necrotizing fasciitis with their characteristic features are as follows:

(a) Necrotizing Cellulitis: Necrotizing cellulitis, or haemolytic *streptococcal* gangrene, usually presents shortly after minor trauma. Patient's findings are consistent with cellulitis, including erythema, warmth, and swelling. Unlike other cellulitis intense pain is common. May also progress to accumulation of gas distal to the wound and blebs containing dark serous fluid. [1]

(b) Streptococcal Myositis: *Streptococcal* myositis typically presents with severe local pain and toxaemia. Wounds present with foul odour, discoloration,

and oedema. Patients are prone to develop blebs and gangrene of the overlying skin, however the disease progression is typically slow. Muscle underneath no longer remains viable, and invariably requires excision. (1)

(c) *Clostridial* Cellulitis: The most crucial historical presentation associated with *clostridial* cellulitis is severe pain which begins days after local tissue injury. This eventually leads to skin blebs filled with reddish brown foul smelling fluid. Cellulitis rapidly progresses within hours and patients become toxic. Though crepitus might be present, it's not a universal finding. (1)

(d)**Progressive Bacterial Synergistic Gangrene or Meleney's Gangrene:** According to Baxter, Progressive Bacterial Synergistic Gangrene (PBSG) and Meleney's ulcer represent variant forms of similar disease process, but has been described as two different entities. In spite of all the differences, clinical profiles were found to be similar.

PBSG is a rapidly progressive infection due to non-haemolytic *Streptococci,* most commonly develops following abdominal surgeries with infected wound and is usually associated with haemolytic *Staphylococci* or gram-negative bacilli. This wound presents with a central necrotic area that is enveloped by purple, erythematous zones of skin. Wounds in addition, possesses necrotic tracts which extends through the underlying tissue, ultimately leading to ulcerations at sites far from the primary lesion.

(e)Fournier's Gangrene: Fournier's gangrene is an acute, rapidly progressive, and potentially fatal, infective necrotizing fasciitis affecting the external

genitalia, perineal, or perianal regions, most common in men although it can occur in women and children. Fournier's Gangrene manifests as sudden pain in the scrotum, prostration, pallor, and pyrexia. Initially only the scrotum is involved, ungoverned, the cellulitis extends till entire scrotal coverings sloughs, leaving the testes exposed but healthy. One imperceptible feature of the presentation is the strong "repulsive, fetid odour" that is associated with this condition. Patients usually present with varying symptoms and signs including fever greater than 38° C, scrotal swelling, erythema, purulent wound discharge, and crepitation. [37]

PREDISPOSING FACTORS AND CO-MORBID CONDITIONS

The entry of pathogens occurs by trauma, insect bite, surgical incision, haematogenous spread from distant sites of infection has also been reported. [38] Some of the other predisposing factors are odontogenic infections, varicella lesions, intramuscular injections and bruises. [39]. NF has been documented even in the absence of any trauma. [40]

NF has even been reported after acupuncture. [41]. This type of specific history is often only obtained if the physician directly questions the patient; otherwise, patients probably ignore or forget to acknowledge.

Non-steroidal anti-inflammatory drug use has been a predisposing aetiology in severe necrotizing streptococcal infections. It is postulated that non-steroidal anti-inflammatory drugs impairs lymphocyte function. (42).Nevertheless it could be due to suppression of symptoms and signs of

inflammation which ultimately leads to delay in diagnosis, particularly in patients presenting early with vague symptoms. [43]

Risk factors for NF in the paediatric population are malnutrition and skin infections such as varicella. [44, 45]. It is necessary to emphasize that physicians should not exclude NF in normal healthy individuals with minor skin trauma. Those are the patients who usually go missed.

RISK FACTORS FOR NECROTIZING FASCIITIS

- Diabetes
- Chronic disease
- Immunosuppressive drugs (eg, prednisolone)
- Age above 60 years
- Malnutrition
- Peripheral vascular disease
- Intravenous drug misuse
- Renal failure
- Underlying malignancy
- Obesity

PRECIPITATING EVENTS CAUSING NECROTIZING FASCIITIS

Table:4

Traumatic	Non traumatic
Surgery	Soft tissue infections
Minor invasive procedures (joint aspiration, accupunture)	Burns
Intravenous drug abuse	Childbirth
Penetrating injuries (insect and animal bites)	

DIAGNOSIS: (11)

The physical appearance and location of lesions within the soft tissues are important diagnostic clues. Other crucial considerations in narrowing down the differential diagnosis are the progression of the lesions as well as the patient's travel history, animal exposure or bite history, age, underlying disease status, and lifestyle .

However, it is difficult to diagnose all infections of the soft tissues by history and inspection alone. Soft tissue radiography, CT and MRI may be useful in determining the depth of infection and should be performed if the patient has rapidly progressing lesions or evidence of a systemic inflammatory response syndrome. These tests are particularly important for defining a localized abscess or detecting gas in tissue.

But they may reveal only soft tissue swelling and hence are not specific for fulminant infections such as necrotizing fasciitis or myonecrosis caused by group A *Streptococcus* where gas may not found in lesions.

Aspiration of the leading edge or punch biopsy with frozen section may also be helpful if the results of imaging tests are positive, however false-negative results occur in ~80% of cases. Aspiration alone may be superior to injection and aspiration with normal saline. Frozen sections are particularly useful in distinguishing *Staphyloccal* Scalded Skin Syndrome (SSSS) from Toxic Epidermal Necrolysis (TEN) and are quite valuable in cases of necrotizing fasciitis.

Laboratory risk indicators of Necrotising Fasciitis (LRINEC) score to discriminate between necrotizing and non-necrotizing soft-tissue infections: [(1)]

Table:5

Value	Score
C-reactive protein, mg/dl <150	0
>150	4
WBC count, cells/mm^3 <15 k	0
15-25 k	1
>25 k	2
Haemoglobin level, g/dl	
>13.5	0
11-13.5	1
<11	2
Sodium level, mmol/L	
>135	0
<135	2
Creatinine level, mg/dl	
<1.6	0
>1.6	2
Glucose level, mg/dl	
<180	0
>180	1

Risk Category based on the LRINEC score:

Table:6

Low	<5	<50% chance of NF
Intermediate	6-7	50-75% chance of NF
High	8	>75% chance of NF

Open surgical inspection, with debridement as indicated, is precisely the best way to determine the extent and severity of infection and to obtain materials for Gram's staining and culture. Such an aggressive approach is necessary for fulminant infections where, associated systemic toxicity is encountered.

MICROBIOLOGY

Type I: This type is usually polymicrobial (aerobes and anaerobes). Enterobacteriaceae are the most common bacteria isolated while *Klebsiella pneumonia, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa* are the common gram negative organisms. In Gram positive *Staphylococcus aureus, Enterococcus species, Streptococcus pyogenes* are common. [46]

Synergistic non-clostridial anaerobic myonecrosis, also known as necrotizing cutaneous myositis and synergistic necrotizing cellulitis, is a variant of necrotizing fasciitis caused by mixed aerobic and anaerobic bacteria with the exclusion of clostridial organisms.

Anaerobes: *Bactroides fragilis*is the most common organism isolated. *Clostridium spp, Peptostreptococcus spp,* are the other organisms found in wounds of NF. *Fusobacterium spp, viellonella parvula, Prevotella brivia, Porphyromonas spp* are the other less common anaerobes causing NF.[46]

Bacteroides: Classified under Gram negative anaerobic non-sporing bacilli. are strict anaerobes and are non-motile. They are pleomorphic, slender rods arranged singly or in pairs or short chains. Bacteroides are normal flora in human intestine.

Bacteroides fragilis: This is the most frequent isolate in clinical specimens. Belongs to the family Bacteroidaceae. More often isolated from wound, pleural fluid, blood, brain abscesses, peritoneal fluid, urogenital infections and soft tissue infections. In general *Bacteroides fragilis* group occur below the diaphragm.

Most of the infection requires breach in mucosal integrity which allows the organisms to gain access to deeper tissues. The hallmark of most but not all infections is the production of foul odour. [47]

Virulence Factors: They produce capsule, endotoxin and succinic acid which inhibit phagocytosis and various enzymes that mediate tissue damage. (47)

IDENTIFICATION [47]

On Anaerobic blood agar: white to grey white, entire convex translucent to semi opaque; non-haemolytic colonies.

On Bacteroides Bile Esculin agar (BBE):colonies are >1mm, circular, entire, and either (a) low convex, dark grey zone (esculin hydrolysis) and sometimes precipitate bile. (b) glistening, convex, light to dark grey and surrounded by grey zone.

Gram Stain: Gram negative, pale- staining, pleomorphic rods with rounded ends; occurs singly or in pairs; often described as safety pin appearance.

Peptostreptococcus: Gram positive anaerobic cocci. Normal flora of the skin, mouth, intestine and vagina. These are recovered from various clinical infections such as puerperal sepsis, skin and soft tissue infections and brain abscess.

PEPTOSTREPTOCOCCUS ANAEROBIUS:[47]

Colonies on anaerobic blood agar: Colonies are grey-white, opaque; sweet, fetid odour; and they are usually larger than most anaerobic cocci.

Gram Stain: Gram-positive, large coccobacillus; often in chains.

Type II: It is a monobacterial, skin or throat derived infection. The causative organism will be either *Group A/B haemolytic streptococci* alone or

in combination with *Staphylococci*. Staphylococci alone can also cause this type of NF. [27]

Group A Streptococci is the most common cause accounting for nearly 60% of total cases of NF. Common serotypes include M types 1 and 3 which produces *Streptococcal* pyrogenic exotoxins.[48]

Type III: Type III includes monomicrobial infections involving the *Clostridium species* which are anaerobic bacteria that enters by external injuries deep wound or crush injury that results in local devascularisation, or surgical wounds particularly intestinal and obstetric. *Clostridium* infections are currently more frequent among drug addicts [49] and *Clostridium perfringens* is the most common bacterium causing type III NF.

Vibrio vulnificus is marine bacterium frequently isolated in Asia. *Vibrio vulnificus* also expresses capsular polysaccharides, toxins, enzymes, metalloproteases, lipopolysaccharides and cytolysin. Shiuan-Chih Chen et al in a retrospective study has documented 89 cases of necrotising fasciitis caused by *Vibrio vulnificus.* [50]

Aeromonas hydrophila is found in fresh water or low salinity water and in the soil. The clinical symptoms caused by these two bacteria are similar.

In a study from Hong Kong showed 83% of their totals NF were type III. [51]. A case of type III NF of the forearm, caused by the *Aeromonas sabori*; had been reported. [27]

TypeIV: These are fungal infections, mainly due to *Candida spp*. and Zygomycetes. This type is found mainly in the immuno compromised host. Infections by these fungi occur after trauma, the clinical image is aggressive and rapidly extensive, and particularly in immuno compromised patients .*Mucor and Rhizopus* are the common Zygomycetes [(27)].

Deepali Jain et al in a retrospective study has reported 18 cases of fungal necrotising fasciitis, out of which five were positive for *Apophysomyces elegans* and fifteen patients were immune competent.[(52)].

Treatment:

Resuscitation and supportive care:

The main objective of resuscitation is to restore adequate perfusion of tissue and oxygen delivery. Invasive arterial pressure monitoring and central venous access may be required; haemodynamic resuscitation in patients presenting with sepsis secondary to NF are as suggested by the Surviving Sepsis Campaign. [(19)]

Adequate nutritional support and treatment of nosocomial infections are important. Critical care admission is strongly recommended in view of the aggressive clinical course, high risk of multi organ failure, and significant mortality rate.

Early and aggressive surgical exploration is essential in cases of suspected necrotizing fasciitis, myositis, or gangrene in order to (1) visualize the deep structures, (2) remove necrotic tissue, (3) reduce compartment

pressure, and (4) obtain suitable material for Gram's staining and for aerobic and anaerobic cultures**.**

Appropriate empirical antibiotic treatment for mixed aerobic–anaerobic infections could consist of ampicillin/sulbactam, cefoxitin, or the following combination: (1) clindamycin (600–900 mg IV every 8 h) or metronidazole (500 mg every 6 h) plus (2) ampicillin or ampicillin/sulbactam (1.5–3g IV every 6 h) plus(3) gentamicin **(**1–1.5 mg/kg every 8h)

Antibiotic treatment should be continued until all signs of systemic toxicity have resolved, all devitalized tissue has been removed, and granulation tissue has developed.[(11)]

Table:7

Condition	**Primary Treatment**	**Alternative treatment**
Necrotising fasciitis (group A *Streptoccocal*)	Clindamycin, 600-900mg IV q6-8h, plus Penicillin G, 4 million units IV q4h.	Clindamycin, 600-900mg IV q6-8h, plus Cephalosporin (first or second generation).
Necrotising Fasciitis (mixed aerobes and anaerobes)	Ampicillin,2g IV q4h, plus, Clindamycin, 600-900 mg IV q6-8h, plus Ciprofloxacin, 400mg IV q6-8h.	Vancomycin, 1g IV q6h, plus, Metronidazole, 500 mg IV q6h, plus Ciprofloxacin, 400mg IV q6-8h.
Gas gangrene	Clindamycin, 600-900mg IV q6-8h, plus Penicillin G, 4 million units IV q4h.	Clindamycin, 600-900mg IV q6-8h, Plus Cefoxitin, 2g IV q6h.

I.V. IMMUNOGLOBULIN THERAPY

The use of i.v. immunoglobulin (IVIG) is based on the mechanism that it can bind staphylococcal- and streptococcal derived exotoxin, thereby reducing the systemic cytokine release which invariably serves as triggering factor for systemic inflammatory response syndrome. There is very limited evidence which suggests a decreased mortality with the use of IVIG in patients of NF due to *Streptococci.* Use of IVIG in NF due to other bacterial aetiology has not been studied. At present use of IVIG is restricted to critically ill patient with either *Staphylococcal* or *Streptococcal* NF. [53,54]

HYPERBARIC OXYGEN (HBO)

Hyperbaric oxygen is one of the preferred treatment for synergistic infections, particularly involving Clostridium spp., hyperbaric oxygen (HBO) switches off toxin production. HBO is considered to enhance the bactericidal action of neutrophils. Nevertheless, the evidence of benefit in *non-Clostridial* NF is poor. Moreover, there are very few hospitals with access to HBO units, appropriate staff, and chambers large enough for patients requiring intensive care support.[55]

Surgical Debridement:

Several studies had documented that the most important factor affecting mortality is timing and adequacy of initial surgical debridement. Delayed or inadequate debridement dramatically increases mortality. [56];

Radical debridement may necessitate limb amputation. Debridement eradicates the source of infection and toxins, and moreover debridement of infarcted tissue eventually improves the penetration of antibiotics. The infection is rarely eradicated after a single debridement and almost always requires serial debridements. Optimally, three debridements at intervals of 12–36 h apart are required to establish control of gross infection. Reconstructive surgery should be considered, when the patient has been stabilized and the infection completely eradicated. Antibiotics cannot penetrate infected necrotic tissue due to the thrombogenic nature of the infection. Hence aggressive surgical debridement remains the first priority. [19]

MATERIALS AND METHODS

ETHICS CONSIDERATION

The study protocol was approved before the commencement of this study, by the Institutional Ethics Committee, Madras Medical College, Rajiv Gandhi Government General Hospital, Chennai. Informed consent was obtained from all the patients included in the study.

STUDY DESIGN

This is a cross-sectional study conducted in the Institute of Microbiology in association with the Institute of General surgery.

DURATION OF THE STUDY

Study duration was one year, from October 2014 to September 2015.

INCLUSION CRITERIA

Patients in the age group 18 years and above were included in the study.

Patients clinically diagnosed as necrotising fasciitis.

EXCLUSION CRITERIA

Patients on antibiotic therapy for one week were excluded.

SAMPLES COLLECTED:

Tissue samples were obtained during wound debridement, (or) wound swabs were obtained from deeper areas of lesion.

These tissue specimens were collected during wound debridement. Swabs were taken from deeper areas of the lesion along the leading edge, after cleaning the wound with sterile saline transported to the laboratory and inoculated in Brain heart infusion broth (BHI) and in Robertson's cooked meat medium (RCM). Two tissue bits (or) two swabs from each patients were collected .

SPECIMEN PROCESSING & METHODOLOGY

Direct Gram Staining was performed for all the specimens collected.

AEROBIC CULTURE

Tissue samples were homogenised and plated onto,

(i)Mac Conkey agar plate

(ii)Blood agar plate and kept in candle jar. Both were incubated at 37ºC overnight and observed for growth.

In Case of wound swabs, they were plated onto

(i) Mac Conkey agar plate,

(ii) Blood agar plates- placed in candle jar and both were incubated at 37ºC, overnight

IDENTIFICATION

Gram's staining was performed from growth obtained.

Gram Positive cocci were initially subjected to

● Catalase test

Catalase positive Gram positive cocci were further subjected to the following standard biochemical and microbiological tests;

(a) Slide and tube coagulase,

(b) Urease test,

(c) Mannitol fermentation test,

(d) Phenolphthalein phosphatase test,

(e) Bacitracin susceptibility test using 0.04U disk.

Catalase negative Gram positive organisms were subjected further to the following biochemical tests;

(a) Bile-esculin hydrolysis,

(b) Heat tolerance test,

(c) Growth in 6.5% bile,

(d) PYR test (pyrolidonyl-beta-naphtylamide),

(e) Carbohydrate fermentation tests using Mannitol, Arabinose, sorbitol, Pyruvate and Raffinose.

Antibiotic susceptibility tests were performed by Kirby Bauer's disk diffusion method on Muller-Hinton agar plate according to CLSI guidelines.

PREPARATION OF INOCULUM FOR SENSITIVITY TESTING

A single colony of the test organism was picked up with sterile loop and suspended in peptone water and incubated at 37ºC for 2hrs. The turbidity of the suspension was adjusted to 0.5 Mac Farland's standard (1.5 x 10^8 CFU/ml). This suspension was spread onto Mueller-Hinton agar plate. A minimum of six antibiotic disks were placed on each MHA plates and were incubated overnight. The zone of inhibition was measured and interpreted as per CLSI (Clinical Laboratory Standards Institute) guidelines.

The control strain used was;

Staphylococcus aureus (ATCC 25923)

Panel of antibiotics included for testing antimicrobial sensitivity of Gram positive cocci.

Table:8

Antibiotics	Disc Content	Zone of Inhibition		
		Sensitive	**Intermediate**	**Resistance**
Penicillin	10 units	≥29	-	≤28
Erythromycin	15µg	≥23	14-22	≤13
Cefoxitin	30µg	≥22	-	≤21
Ciprofloxacin	5µg	≥21	16-20	≤15
Co-trimoxozole	1.25/23.75µg	≥16	11-15	≤10
Amikacin	30µg	≥17	15-16	≤14
High level gentamicin	120µg	≥10	7-9	≤6
Chloromphenicol	30µg	≥18	13-17	≤12

Stapylococcus aureus strains were screened for Methicillin resistance.

DETECTION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

CEFOXITIN DISC METHOD[53]

Colonies of *Staphylococcus aureus*(test isolate) were inoculated in peptone water and matched with 0.5 Mac Farland standard. The suspension was streaked onto cation adjusted Muller-Hinton agar plates. And Staphylococcus aureus ATCC 25923 (Control) was also cultured onto another separate cation adjusted MHA. Cefoxitin disc (30 µg) was placed on the lawn culture of both the test and control isolates and incubated at 33-35ºC overnight.

INTERPRETATION

As per **CLSI** guidelines,

Zone of inhibition: ≥22mm (mec A negative)

Zone of inhibition: ≤21mm (mec A positive)

Cefoxitin is used as a surrogate marker for mec A- mediated oxacillin resistance. Isolates that test as mec A positive should be reported as oxacillin (not cefoxitin) resistant.

MRSA isolates were to Minimum Inhibitory Concentration by E-test method.

E-TEST (EPSILOMETER TEST): VANCOMYCIN EZY MIX STRIP VAN, HI-MEDIA

It is a unique Minimum Inhibitory Concentration determination paper strip coated with Vancomycin in a predefined quantitative gradient. MIC can be determined in the range of 0.016mcg/ml to 256mcg/ml, on testing against the test organism.

QUALITY CONTROL

ATCC *Staphylococcus aureus* 25923.

PROCEDURE

MRSA strains of Staphylococcus aureus were inoculated in peptone water and incubated for two hours at 37ºC and then streaked onto cation adjusted Mueller-Hinton agar plates using a sterile cotton swab.

Ezy MIC strips were brought to room temperature 15 minutes before use. Using an applicator provided in the kit bag, the Ezy MIC strip was placed on the plate seeded with the MRSA strain. The plates were then incubated at 33º-35ºC overnight.

Control strain of ATCC *Staphylococcus aureus* 25923 was also tested similarly.

INTERPRETATION

Sensitive-2µg,

Intermediate-4-8µg,

Resistant- >16µg.

GRAM NEGATIVE ORGANISMS

The following preliminary and standard biochemical tests were performed;

(a) Catalase test,

(b) Oxidase test,

(c) Motility by hanging drop method,

(d) Indole test,

(e) Methyl red test,

(f) Voges proskeur test,

(g) Citrate utilization test,

(h) Urease hydrolysis test,

(i) Hugh-leifsons of test,

(j) Mannitol motility test

(k) Lysine decarboxylase, Ornithine decarboxylase, Arginine dihydrolase,tests

(l) Carbohydrate fermentation tests using Glucose, Sucrose, Lactose, Maltose, Mannose fermentation tests were also performed.

*Acinetobacter baumanni*was speciated by the following tests;

(a) Growth at 44ºC,

(b) Presence of β-hemolysis,

(c) 10% OF lactose utilisation test,

(d) Malonate utilisation test.

Antibiotic susceptibility tests were performed by Kirby Bauer's disk diffusion method on Mueller- Hinton agar plate according to CLSI guidelines.

The following organisms were used as controls:

(1) *Escherichia coli* (ATCC 25922),

(2) *Pseudomonas aeruginosa* (ATCC 27853)

PANEL OF ANTIBIOTICS INCLUDED FOR TESTING ANTIMICROBIAL SENSITIVITYOF GRAM NEGATIVEBACILLI

Table:9

Antibiotics	Disk content	Gram negative bacilli	Diameter of zone of inhibition in mm		
			Sensitive	Intermediate	Resistant
Amikacin	30µg		≥17	15-16	≤14
Gentamicin	10µg		≥15	13-14	≤12
Ciprofloxacin	5µg		≥21	18-20	≤17
Cotrimoxozole	1.25/23.75 µg		≥16	11-15	≤10
Cefotaxime	30µg	Enterbacteiaceae	≥26	23-25	≤22
		Acinetobacter sp.	≥23	15-22	≤14
Ceftazidime	30µg	Enterbacteiaceae	≥21	18-20	≤17
		P.aeruginosa& *Acinetobacter sp.*	≥18	15-17	≤14
Imipenem	10µg	Enterbacteiaceae	≥23	20-22	≤19
		P.aeruginosa	≥19	16-18	≤15
		Acinetobacter sp.	≥16	14-15	≤13
Piperacilin-Tazobactum	100/10µg		≥21	18-20	≤17

Gram negative organisms which were resistant to cefotaxime/ceftazidime were subjected for confirmatory test to detect ESBL production by combination disk method.

PHENOTYPIC ESBL CONFIRMATORY TEST

ANTIBIOTICS USED

(1) Cefotaxime (30µg)

(2) Cefotaxime/Clavulunate (30/10µg)

(3) Ceftazidime (30µg)

(4) Ceftazidime/Clavulunic acid (30/10µg)

DETECTION OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCTION

Using sterile loop five to six colonies of similar morphology of the test organism were picked up and inoculated in peptone water and incubated at 37ºC for 2-4hrs and then the suspension was matched with 0.5 Mac Farland standard ($1.5x10^8$cfu/ml). Lawn culture was performed on MHA plate. Cefotaxime (30 µg), cefotaxime/clavulanate (30/10 µg) and Ceftazidime (30 µg), Ceftazidime/clavulanate (30/10 µg) disks were placed on the plate with 24mm gap between each disks and incubated at 37ºC overnight.

INTERPRETATION:[53]

A zone difference of ≥ 5mm around the Cefazidime/Ceftazidime-Clavulanate disks than Ceftazidime and a zone difference of ≥3mm around cefotaxime disks than Cefotaxime/Clavulanate is phenotypical confirmed to be due to ESBL production.

ANAEROBIC CULTURE: [48]

Wound swabs or tissue samples were collected and inoculated in Robertson's cooked meat medium and then were transported to the laboratory.

Tissue specimens or swabs inoculated in RCM were plated onto anaerobic blood agar plates (blood agar with gentamicin and vitamin K). These plates were placed inside a Macintosh jar, along with a blood agar plate on which Pseudomonas aeruginosa was streaked as control. Anaerogas was kept inside the Macintosh jar, then closed and sealed with petroleum jelly and incubated at 37ºC for 48-72hrs and observed for growth.

Growth obtained were subjected to Gram staining.

Gram stain exhibiting Gram positive cocci, were initially subjected to,

Catalase test

The isolate were then, sub cultured onto anaerobic blood agar plate and the following presumptive antibiotic identification disks were placed on the plate.

Kanamycin, (1mg)

Colistin, (10 µg)

Vancomycin, (5µg) disks.

Also sodium polyantheol sulfonate (SPS) was placed near colistin disk. This SPS disk was used for rapid identification of *Peptostreptococcus*

anaerobius, growth which is inhibited by SPS. *Pseudomonas aeruginosa* was streaked onto another blood agar plate as control. The plates were incubated anaerobically in Macintosh jar with Anaerogas at 37ºC for 48-72hrs.

Gram stain showing Gram negative bacilli were similarly subjected to catalase test and sub cultured onto anaerobic blood agar plate and the same identification disks were used. In addition to the other disks, nitrate disk was also placed and incubated anaerobically in Macintosh jar with Anaerogas at 37ºC for 48-72hrs.

Growth obtained were also tested for aerotolerance. Colonies were streaked onto chocolate agar plate and incubated in 5-10% of carbon dioxide at 37ºC overnight. Since there was no growth observed in these plates the isolates were considered to be obligate anaerobes.

Identification of Anaerobes: [48]

Table:10

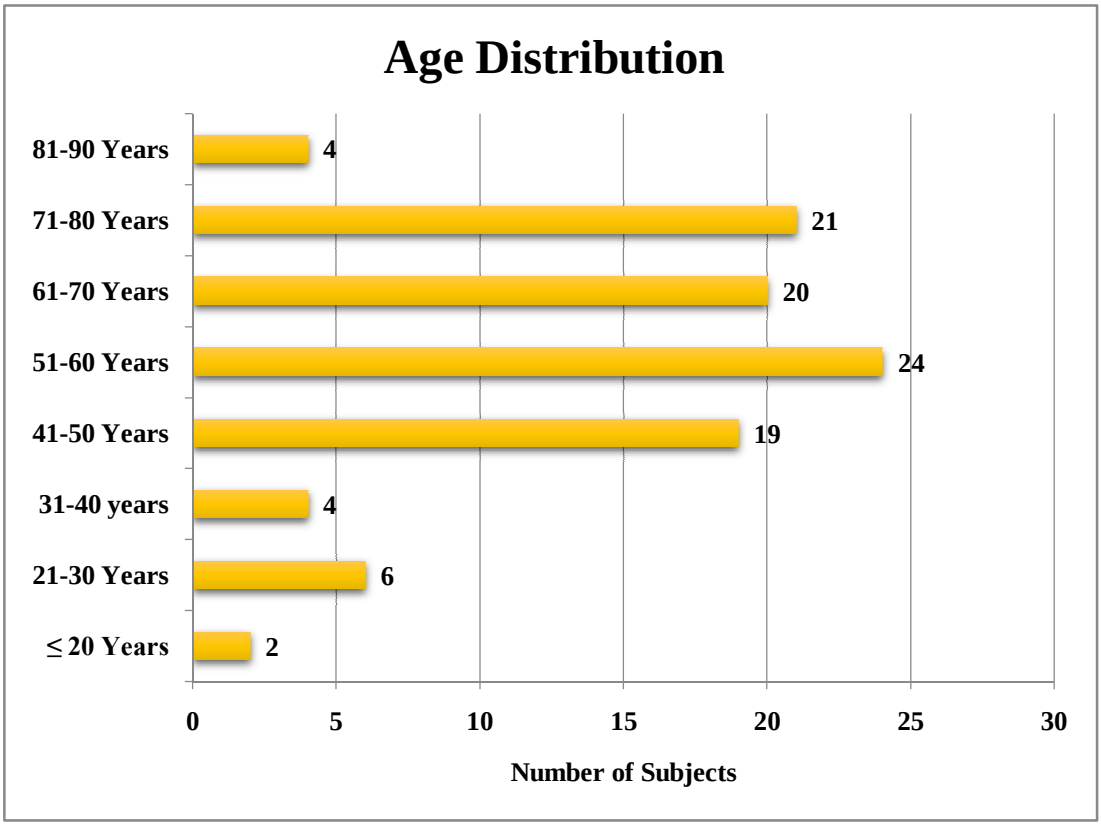
Organisms	Cell shape	Kanamycin(1 mg)	Vancomycin(5µg)	Colistin (10 mg)	Nitrate	SPS	Catalase
Bacteroides fragilis	Bacilli	R	R	R	-	-	-Ve
Peptostreptococcus anaerobius	Cocci	R	S	R	-	S	-Ve

RESULTS

This cross-sectional study was conducted at the Institute of Microbiology in association with the Institute of General Surgery at Madras Medical College, RGGGH, Chennai. Duration of the study was one year, from October 2014 to September 2015. Study population included one hundred patients with Necrotising Fasciitis.

Age Distribution among the study population: (n=100)

Figure 2



Age Distribution among the study population: (n=100)

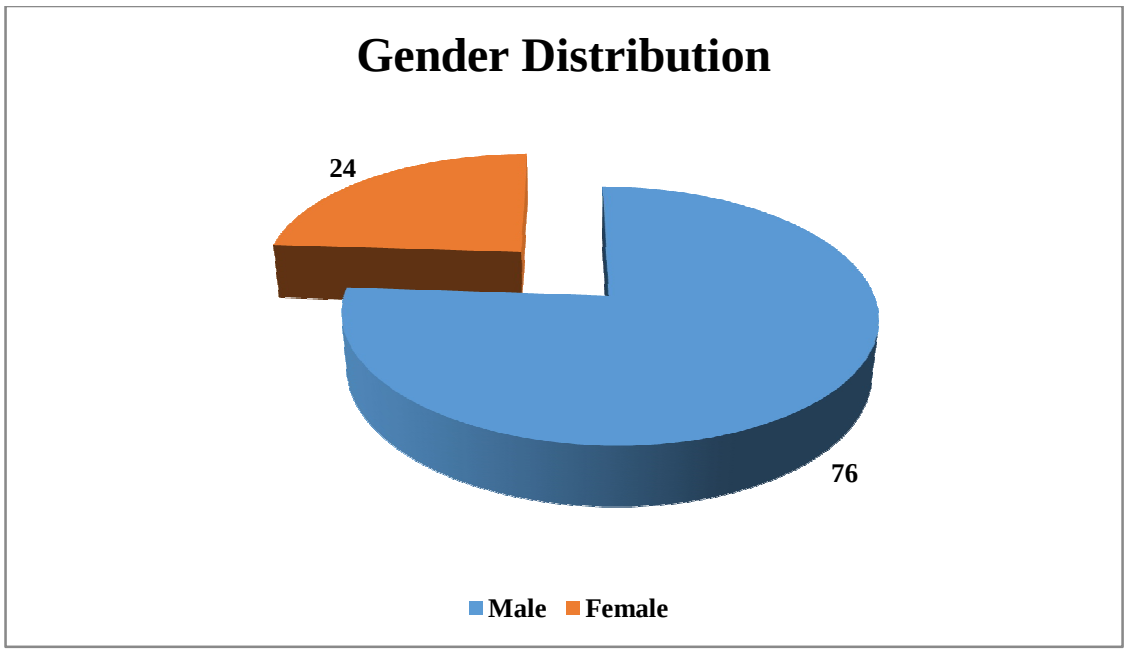
Table:11

Age Distribution (n=100)	Frequency	Percentage
≤ 20 Years	2	2
23 – 30 Years	6	6
31-40 Years	4	4
41-50 Years	19	19
51-60 Years	24	24
61-70 Years	20	20
71-80 Years	21	21
81-90 Years	4	4
P value One Smaple t- Test		0.4431

Majority of the patients belonged to the age group of 51-60 years of age (n=24, 24%) with a mean age of 58.35 years. The minimum age was 18 years and the maximum was 86 years. Since p value is > 0.05 as per one sample t test, the age distribution was not considered statistically significant.

Gender Distribution of the study population: (n=100)

Figure 3



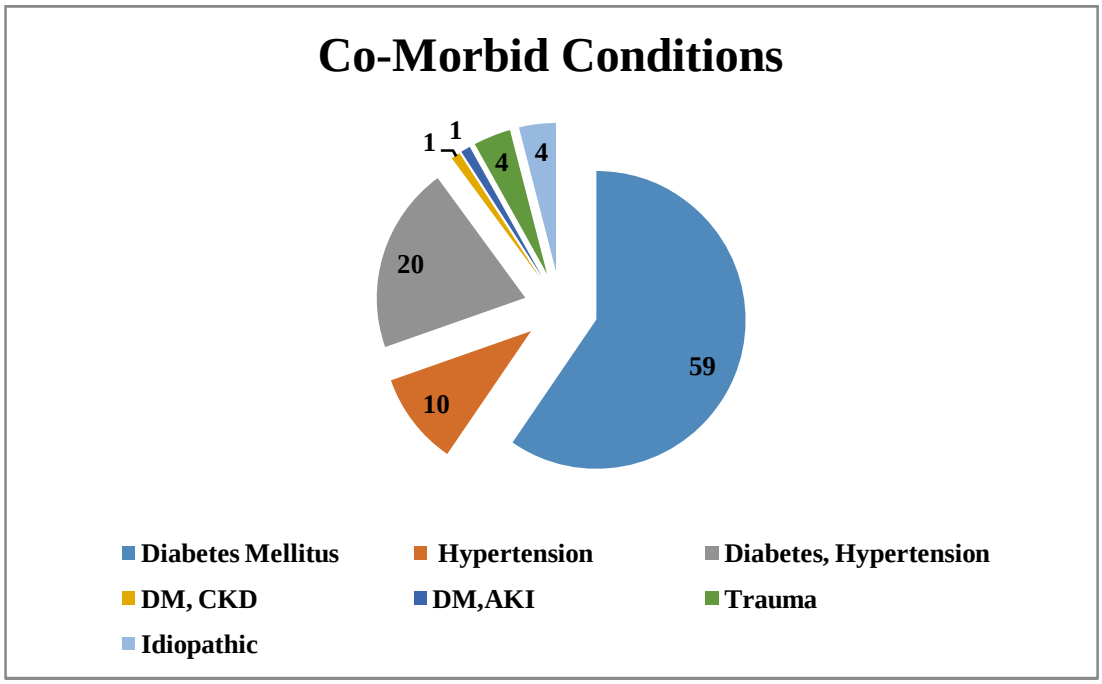
Gender Distribution of the study population: (n=100)

Table:12

Gender Distribution	Frequency	Percentage
Male	76	76
Female	24	24
Total	100	100
P value One Sample Z-Test		0.0044

Majority of the patients were male (n=76, 76%) and the rest were females (n=24, 24%). By conventional criteria gender distribution is taken as statistically significant as the P-value (0.0044) was lower than the significance level (0.05) by one sample Z test.

Figure 4



Co-Morbid Conditions: (n=100)

Table:13

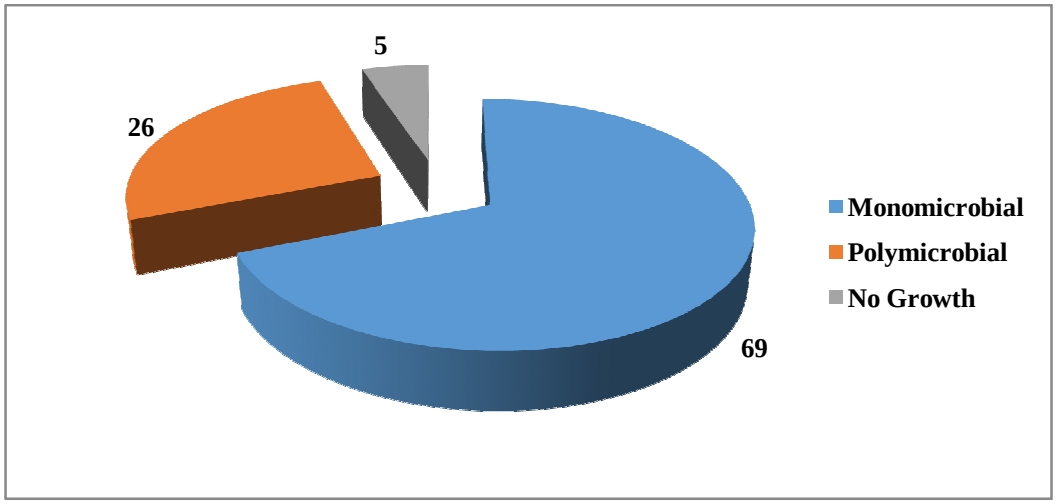
Co-morbidity (n=100)	Frequency	Percentage
Idiopathic	5	5
DM	59	59
HT	10	10
DM + HT	20	20
DM + CKD	1	1
DM + AKI	1	1
Trauma	4	4
Total	100	100
P value One Sample Z-Test		0.0001

Type II Diabetes Mellitus was found to be the commonest co-morbid condition (n=59, 59%) in patients of NF. Using one sample Z test P- value was

determined as 0.0001 and was found to be lower than 0.05, hence association of type II DM is considered statistically significant in patients of NF.

Frequency of Isolates based on Microbiological classification:

Figure 5



Frequency of Isolates based on Microbiological classification: (n=95)

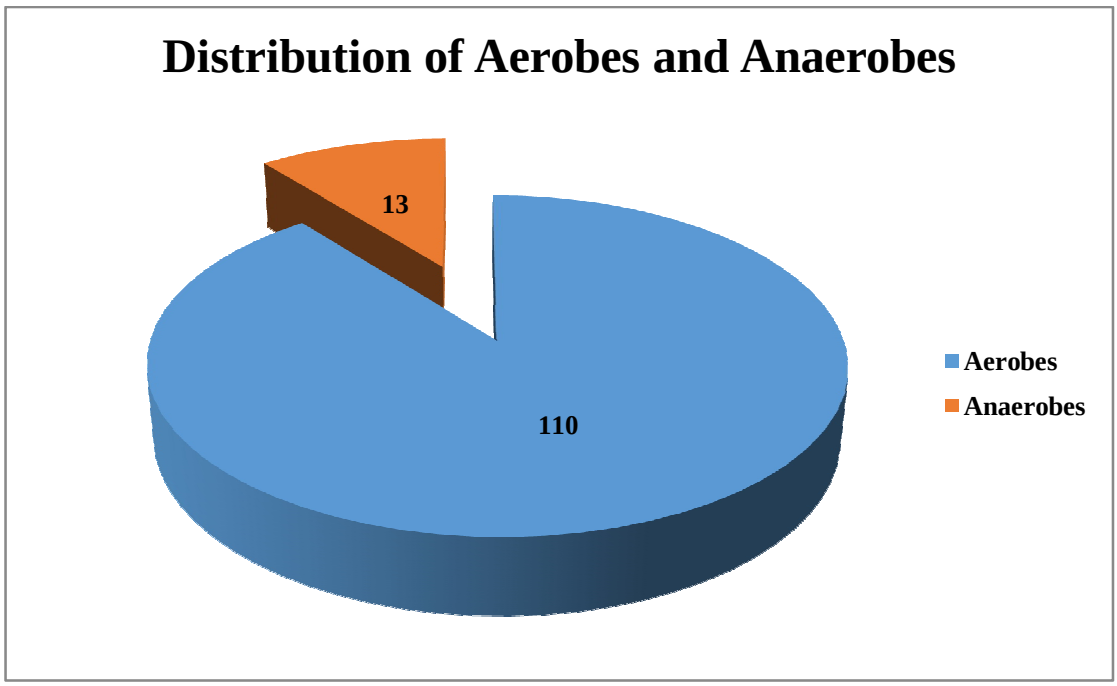
Table:14

Microbial Isolates (n=95)	Frequency	Percentage
Monomicrobial	69	72.63
Polymicrobial	26	27.34
Total	100	100
P value One Sample Z-Test		0.0414

Monomicrobial isolates (n=69, 69%) were more frequent than polymicrobial isolates (n=26, 26%). The distribution of monomicrbial isolates

is considered statistically significant since the one sample Z test showed P-value of 0.0414 which was lower than the significant level (0.005).

Figure 6

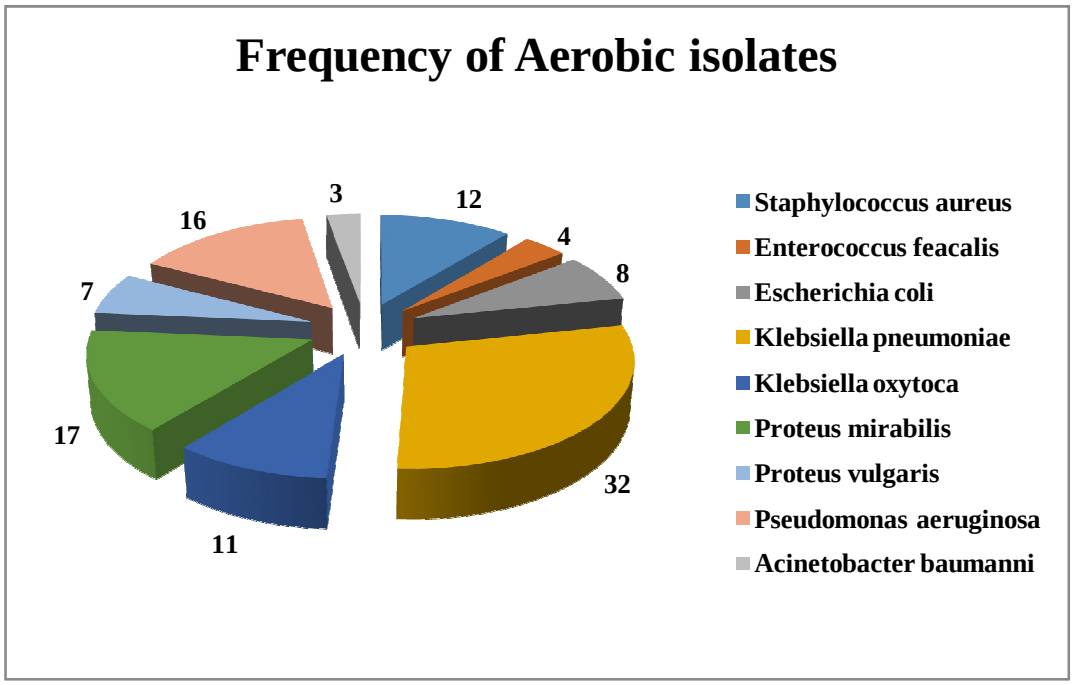


Distribution of Aerobes and Anaerobes: (n=123)

Table:15

Organisms Isolated (n=123)	Frequency	Percentage
Aerobes	110	89.43
Anaerobes	13	10.57
Total	123	100

Figure 7

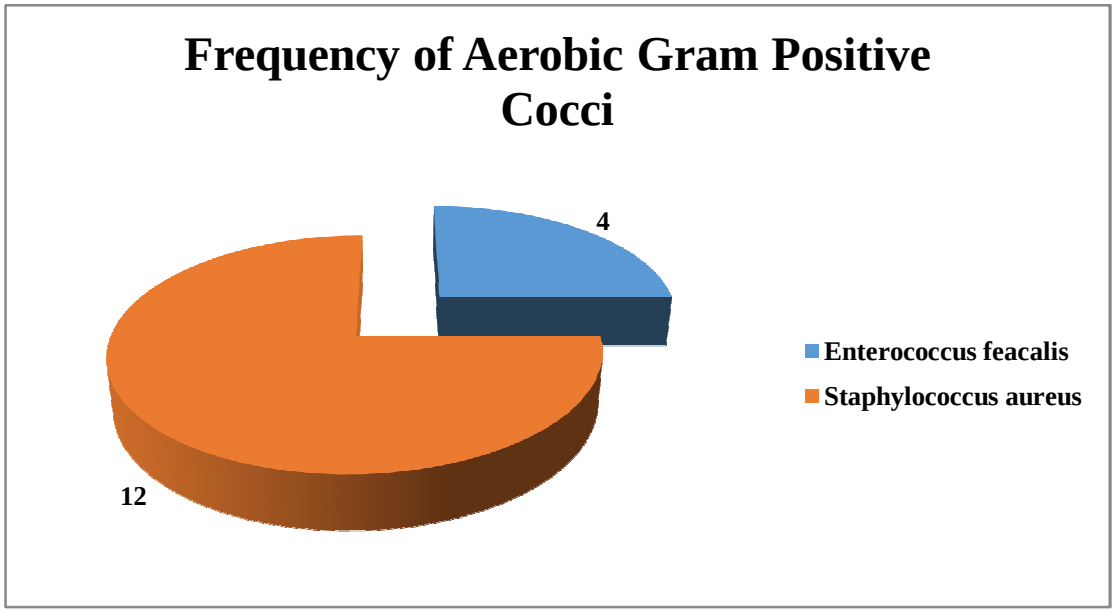


Frequency Of Aerobic Isolates: (n=110)

Table:16

Organisms isolated	Frequency	Percentage
Staphylococcus aureus	12	10.9
Enterococcus feacalis	4	3.63
Escherichia coli	8	7.27
Klebsiella pneumonia	32	29.09
Klebsiella oxytoca	11	10
Proteus mirabilis	17	15.45
Proteus vulgaris	7	6.36
Pseudomonas aeruginosa	16	14.54
Acinetobacter baumanni	3	2.72
Total	110	100
P value One Sample Z - Test		0.0081

Figure 8

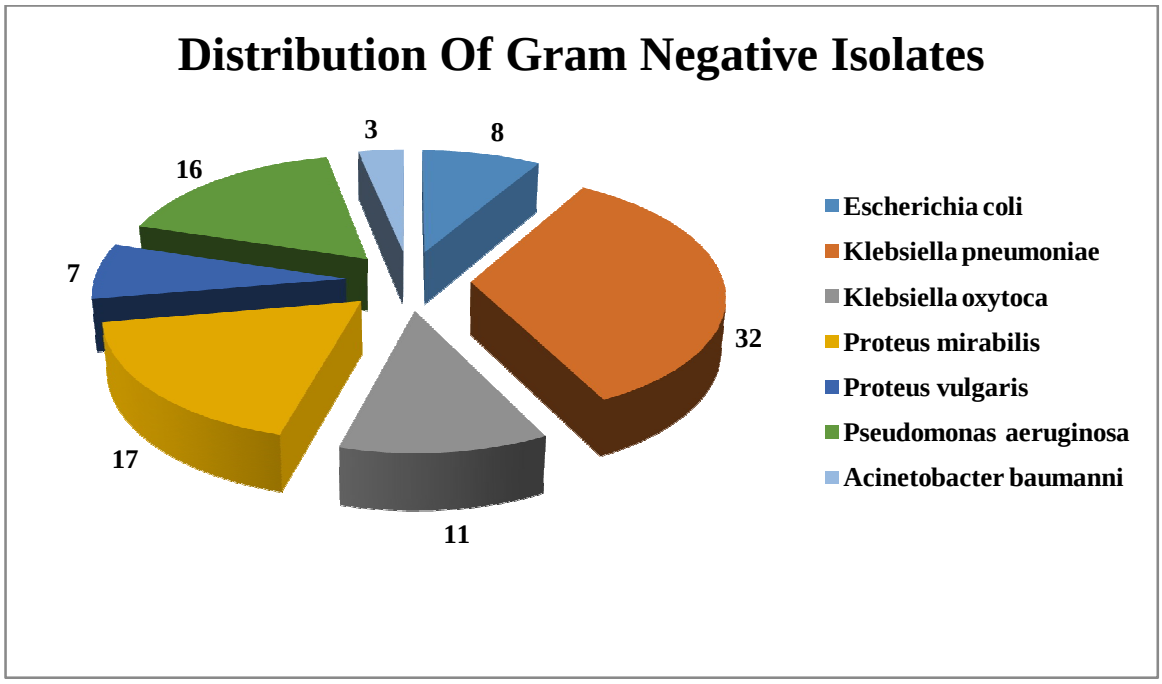


Frequency Of aerobic Gram positive cocci isolated:(n=16)

Table:17

Gram Positive Cocci (n=16)	No.of Isolates	%
Enterococcus feacalis	4	25.00
Staphylococcus aureus	12	75.00
Total	16	100

Figure 9

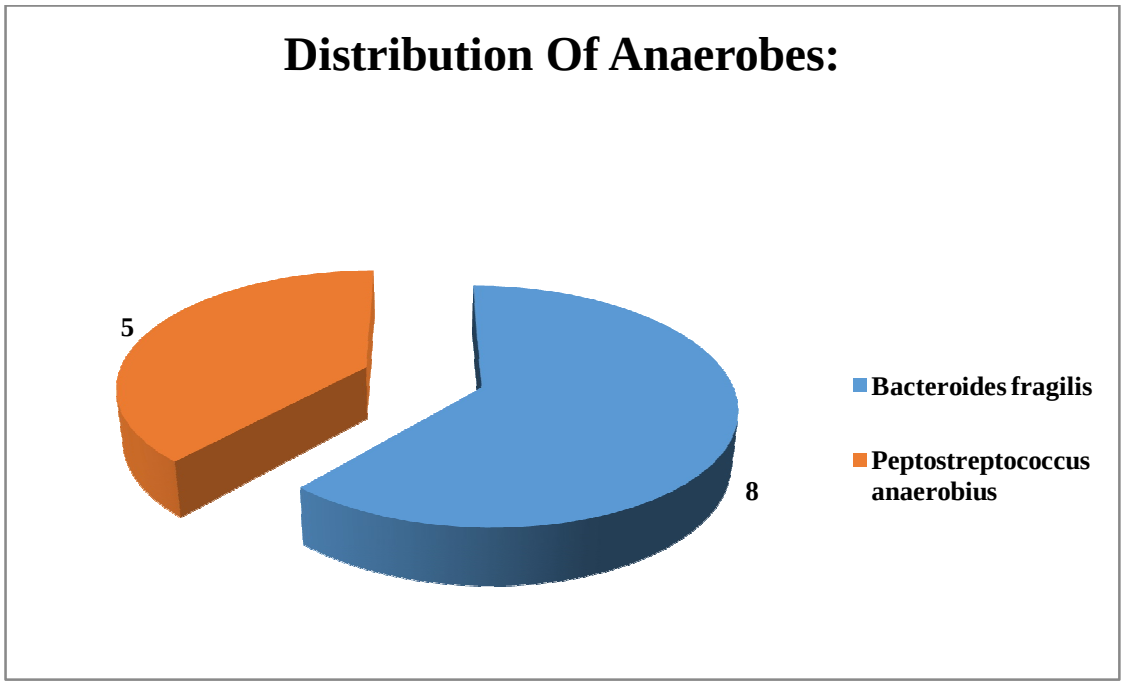


Frequency Of aerobic Gram negative organisms Isolated: (n=94)

Table:18

Gram negative Isolates (n=94)	Frequency	Percentage
Escherichia coli	8	8.51
Klebsiella pneumonia	32	34.04
Klebsiella oxytoca	11	11.70
Proteus mirabilis	17	18.09
Proteus vulgaris	7	7.45
Pseudomonas aeruginosa	16	17.02
Acinetobacter baumanni	3	3.19
Total	94	100

Figure 10

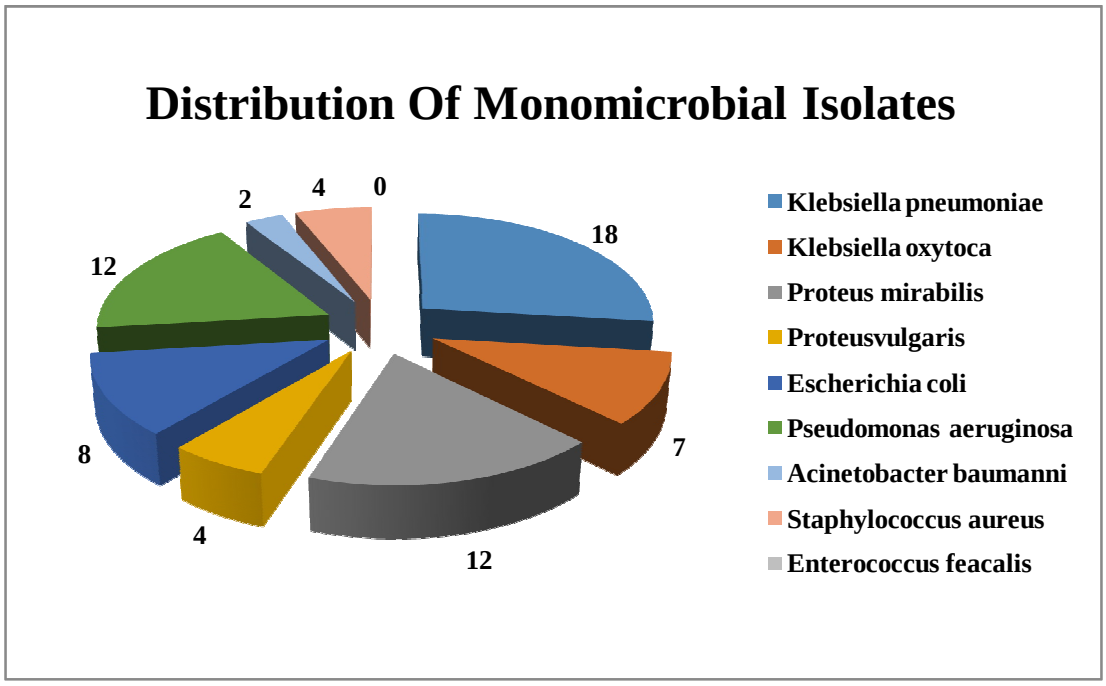


Distribution Of Anaerobes: (n=13)

Table:19

Anaerobes Isolated (n=13)	Frequency	Percentage
Bacteroides fragilis	8	61.54
Peptostreptococcus anaerobius	5	38.46
Total	13	100

Figure 11

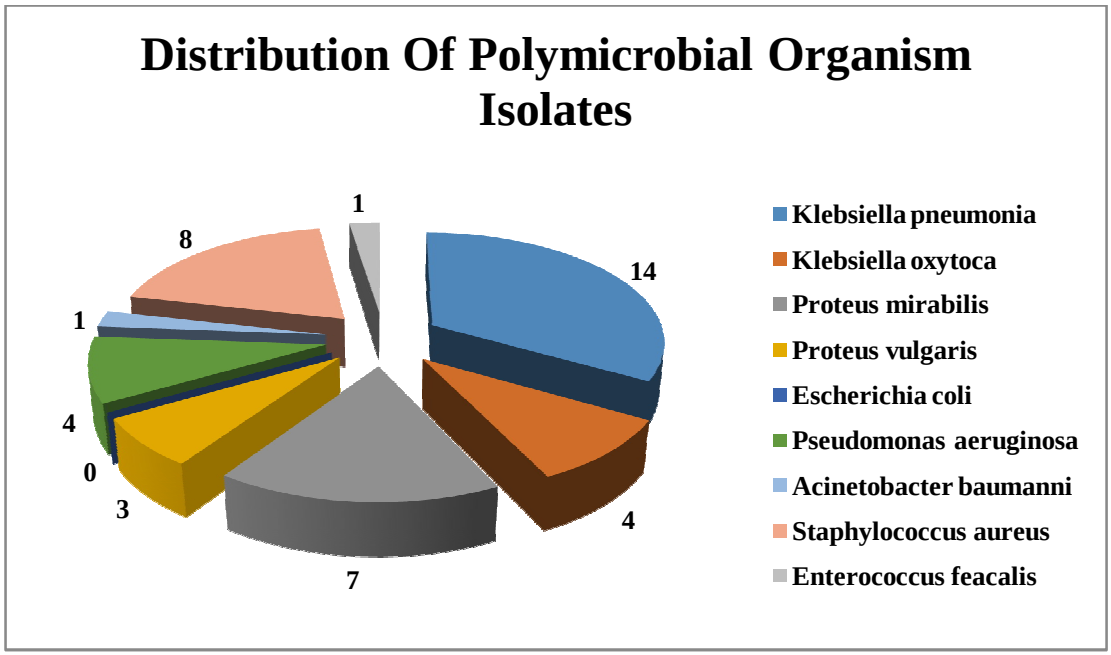


Distribution Of Monomicrobial Isolates: (n=69)

Table:20

Monomicrobial Organism Isolated (n=69)	**Frequency**	**Percentage**
Klebsiella pneumoniae	18	26.09
Klebsiella oxytoca	7	10.14
Proteus mirabilis	12	17.39
Proteus vulgaris	4	5.80
Escherichia coli	8	11.59
Pseudomonas aeruginosa	12	17.39
Acinetobacter baumanni	2	2.90
Staphylococcus aureus	4	5.80
Enterococcus feacalis	2	2.90
Total	69	100

Figure 12



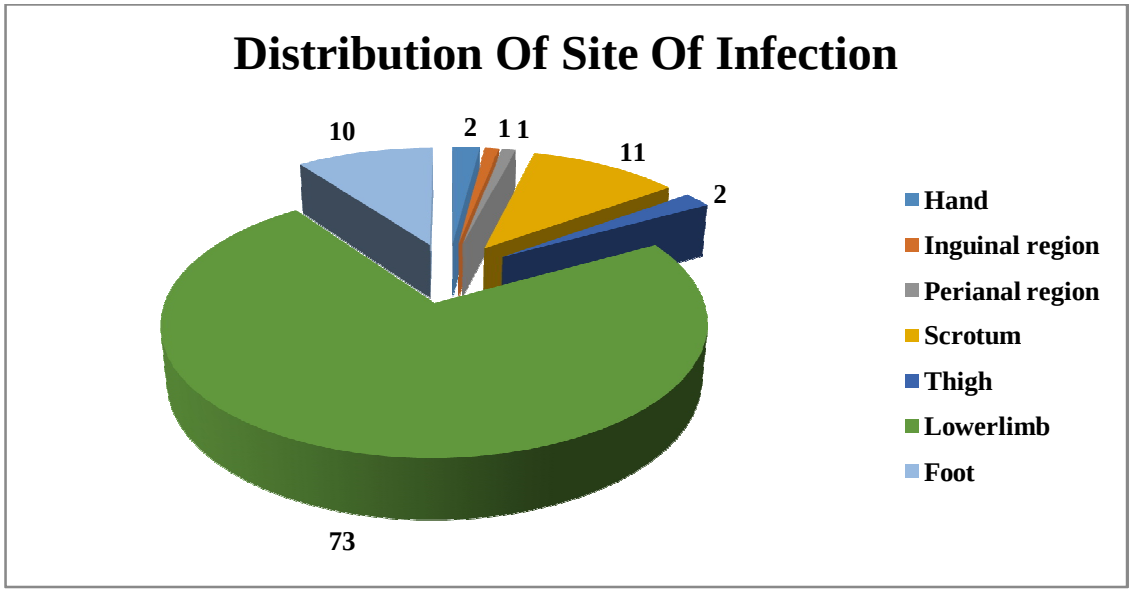
Distribution Of Polymicrobial Isolates: (n=42)

Table:21

Polymicrobial Organism Isolated (n=42)	**Frequency**	**Percentage**
Klebsiella pneumoniae	14	33.33
Klebsiella oxytoca	4	9.52
Proteus mirabilis	7	16.67
Proteus vulgaris	3	7.14
Escherichia coli	0	0.00
Pseudomonas aeruginosa	4	9.52
Acinetobacter baumanni	1	2.38
Staphylococcus aureus	8	19.05
Enterococcus feacalis	1	2.38
Total	42	100
P Value One Sample Z- test	110	0.0081

Klebsiella pneumoniae was the most common organism isolated in both monomicrobial (n=18, 26.08%) and polymicrobial (n=14, 33.33%) isolates. Distribution of *Klebsiella pneumoniae* showed a P-value of 0.0081 by one sample Z test, and is taken as statistically significant.

Figure 13



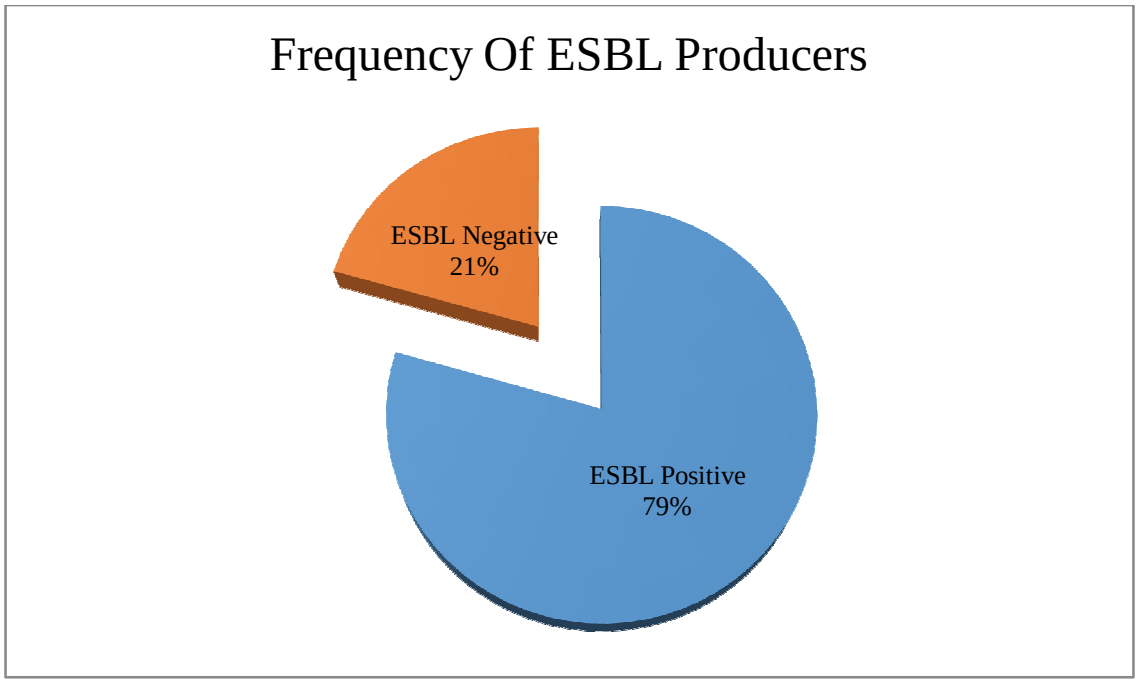
Frequency Distribution Of Site Of Infection: (n=100)

Table:22

Site of infection (n=100)	Frequency	Percentage
Hand	2	2.00
Inguinal Region	1	1.00
Perianal	1	1.00
Perineum	11	11.00
Thigh	2	2.00
Lower limb	73	73.00
Foot	10	10.00
Total	100	100
P value One Sample Z-Test		0.0133

Lower limb (n=73, 73%) was the most common site affected in the study population. P-value by one sample Z test was 0.0133 (< 0.05). Hence it is considered as statistically significant.

Figure 14



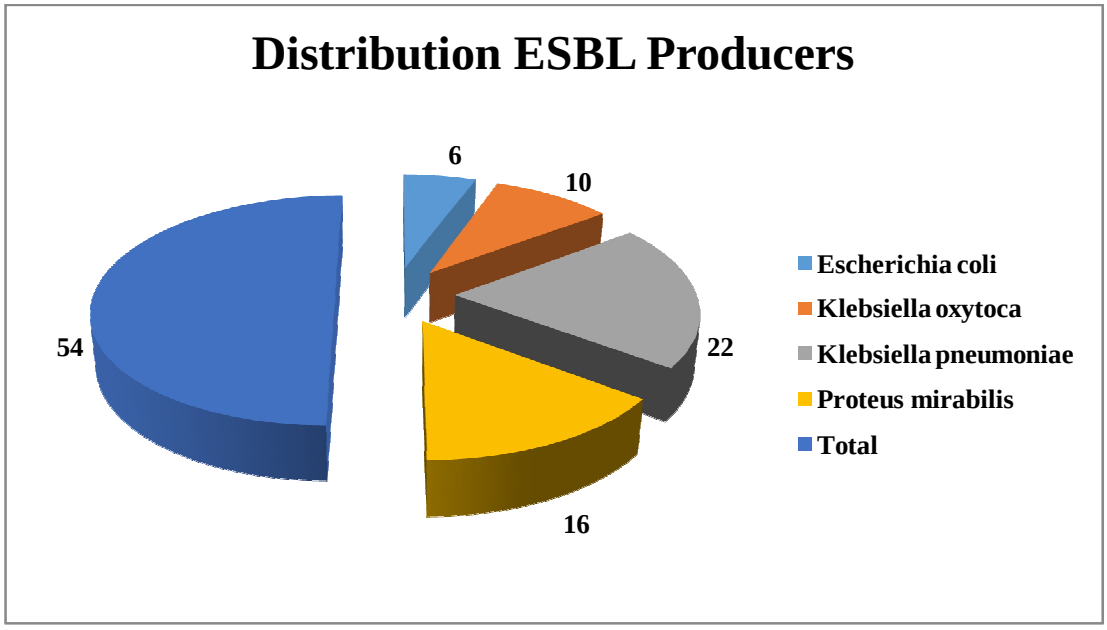
Frequency Of Extended Spectrum Beta Lactamase (ESBL) Producers:

(n=68)

Table:23

ESBL Production (n=68)	Frequency	Percentage
Present	54	79
Absent	14	21
Total	68	100
P-value One Sample Z test		0.0310

Figure 15



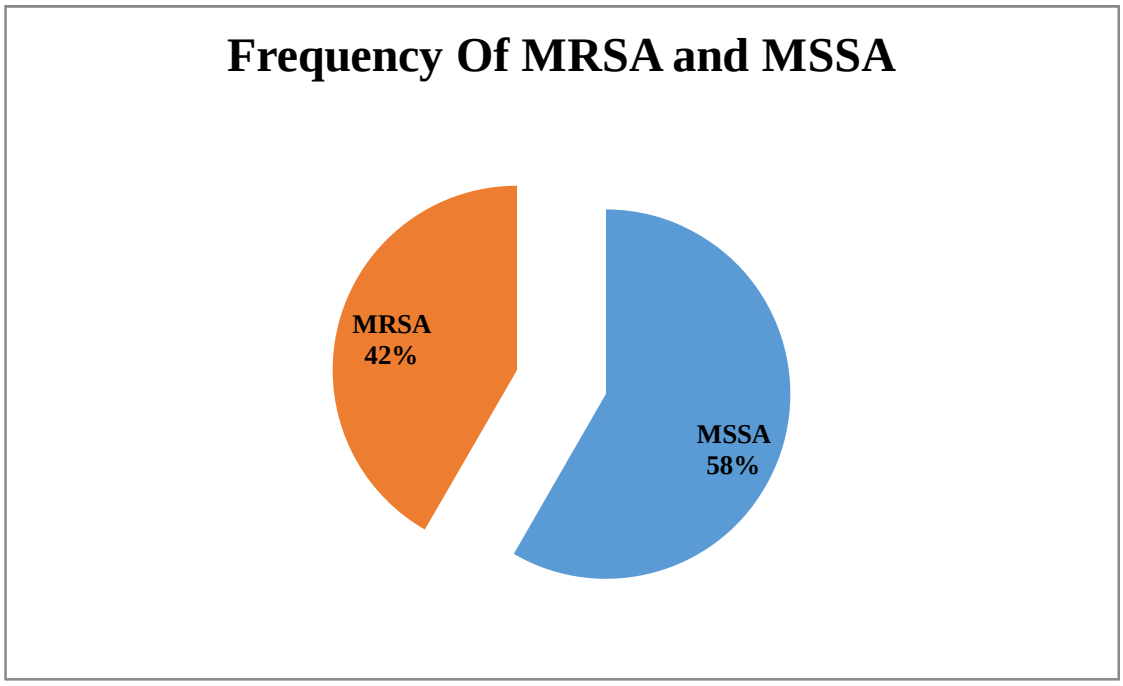
Distribution Of ESBL Producers: (n=54)

Table:24

ESBL	ESBL Positive	Percentage
Escherichia coli	6	11.11
Klebsiella oxytoca	10	18.52
Klebsiella pneumoniae	22	40.74
Proteus mirabilis	16	29.63
Total	54	100

Klebsiella pneumoniae (n=22, 40.74%) contributed to most of ESBL isolates. By one sample Z test p-Value was 0.0310 which was lower than the significance level 0.05, hence this is considered to be statistically significant.

Figure 16



Frequency Of MRSA and MSSA: (n=12)

Table:25

***Staphylococcus aureus* (n=12)**	**Frequency**	**Percentage**
MRSA	5	41.67
MSSA	7	58.33
Total	12	100
P value One Sample Z-Test		0.0169

Of the 12 *Staphylococcus aureus* isolates, n=5, 41.67% were MRSA and n=7, 58.33% were MSSA, Using one sample Z test P-value was calculated. Since it was 0.0169, it is statistically significant.

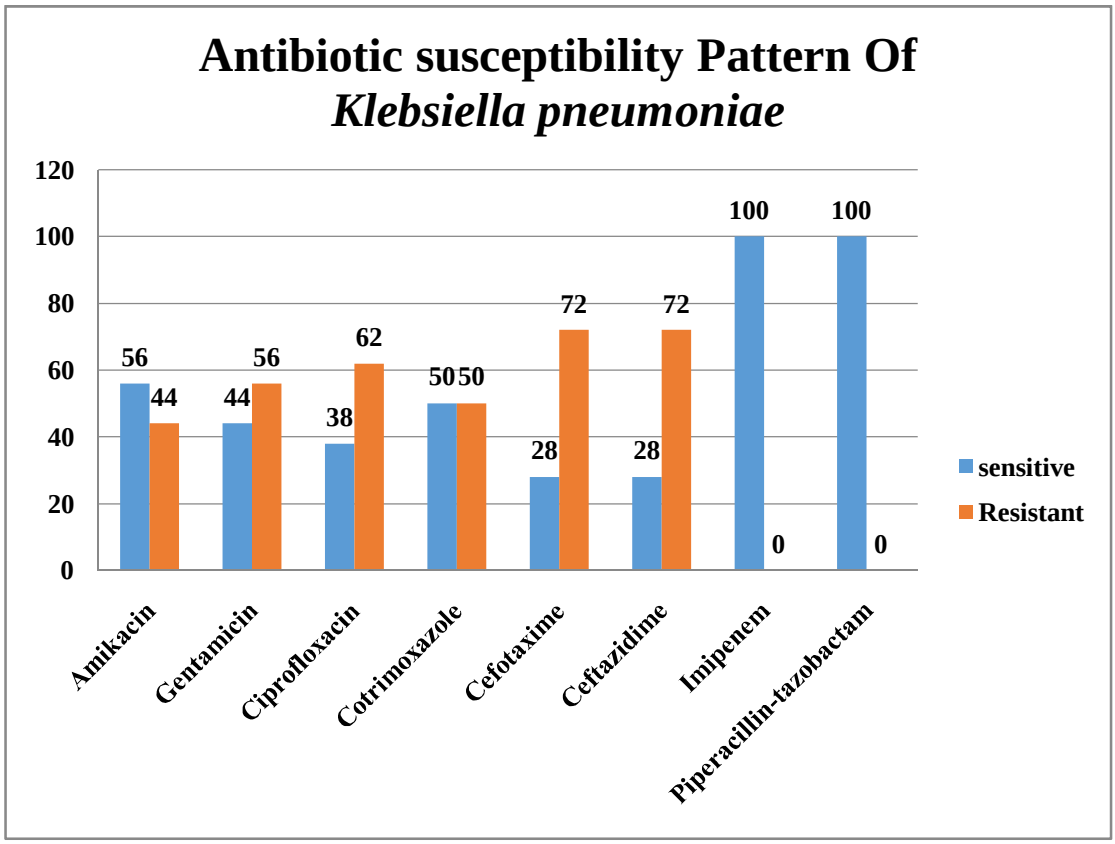
Vancomycin Sensitivity OF MRSA isolates: (n=5)

Table:26

Vancomycin	**MRSA**
Sensitive (MIC <2µg)	**5**
Intermediate (MIC 4-8µg)	**0**
Resistant (MIC 16µg)	**0**
Total	**5**

All the MRSA isolates were sensitive to vancomycin by E-test.

Figure 17

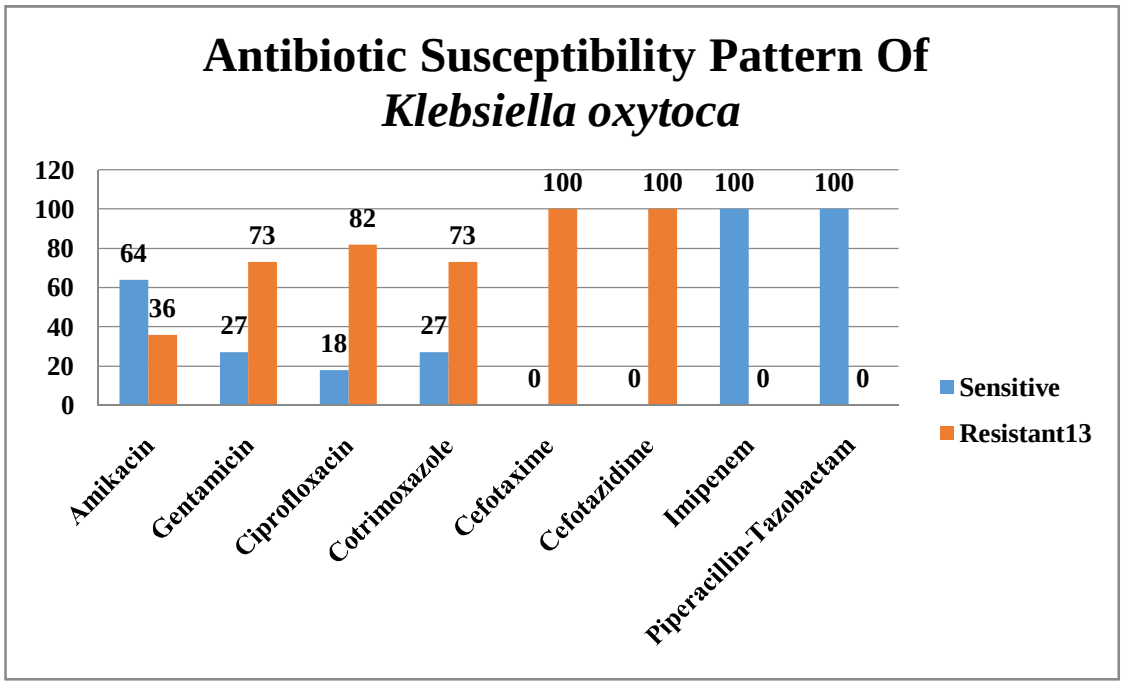


Antibiotic Susceptibility Pattern of *Klebsiella pneumoniae*: (n=32)

Table:27

Antibiotic Susceptibility - *Klebsiella pneumonia* (n=32)	**Sensitive (%)**	**Resistant (%)**
Amikacin	56	44
Gentamicin	44	56
Ciprofloxacin	38	62
Cefotaxime	28	72
Ceftazidime	28	72
Cotrimoxozole	50	50
Imipenem	100	0
Piperacillin-Tazobactam	100	0

Figure 18

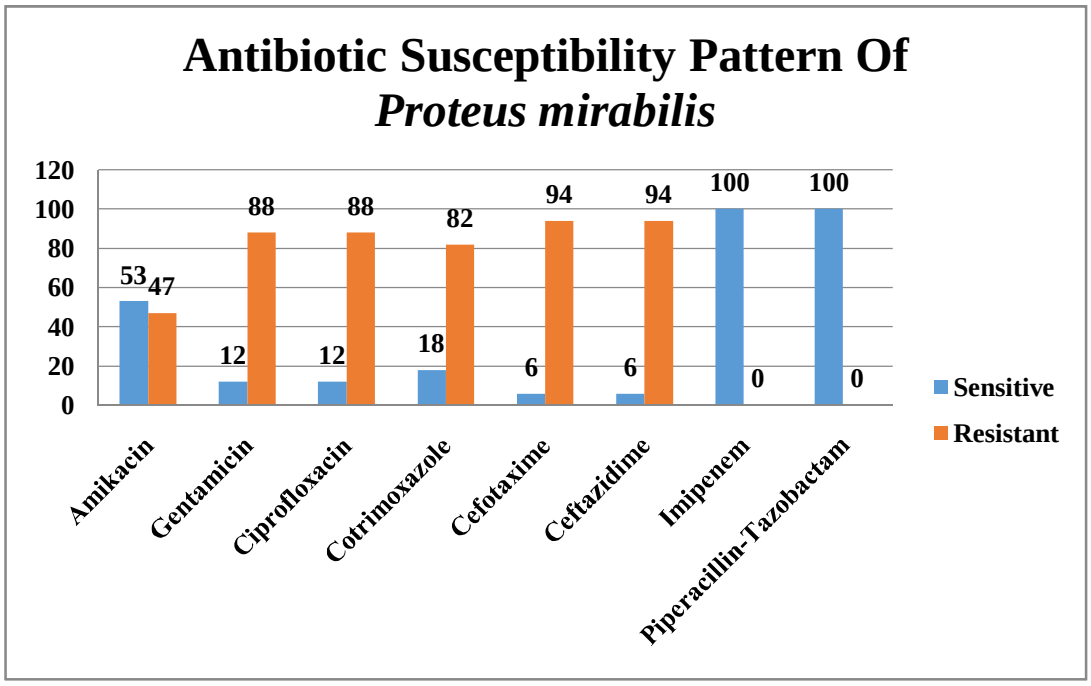


Antibiotic Susceptibility Pattern of *Klebsiella oxytoca*: (n=11)

Table:28

Antibiotic Susceptibility - *Klebsiella oxytoca* (n=11)	**Sensitive (%)**	**Resistant (%)**
Amikacin	64	36
Gentamicin	27	73
Ciprofloxacin	18	82
Cefotaxime	0	100
Ceftazidime	0	100
Cotrimoxozole	27	73
Imipenem	100	0
Piperacillin-Tazobactam	100	0

Figure 19

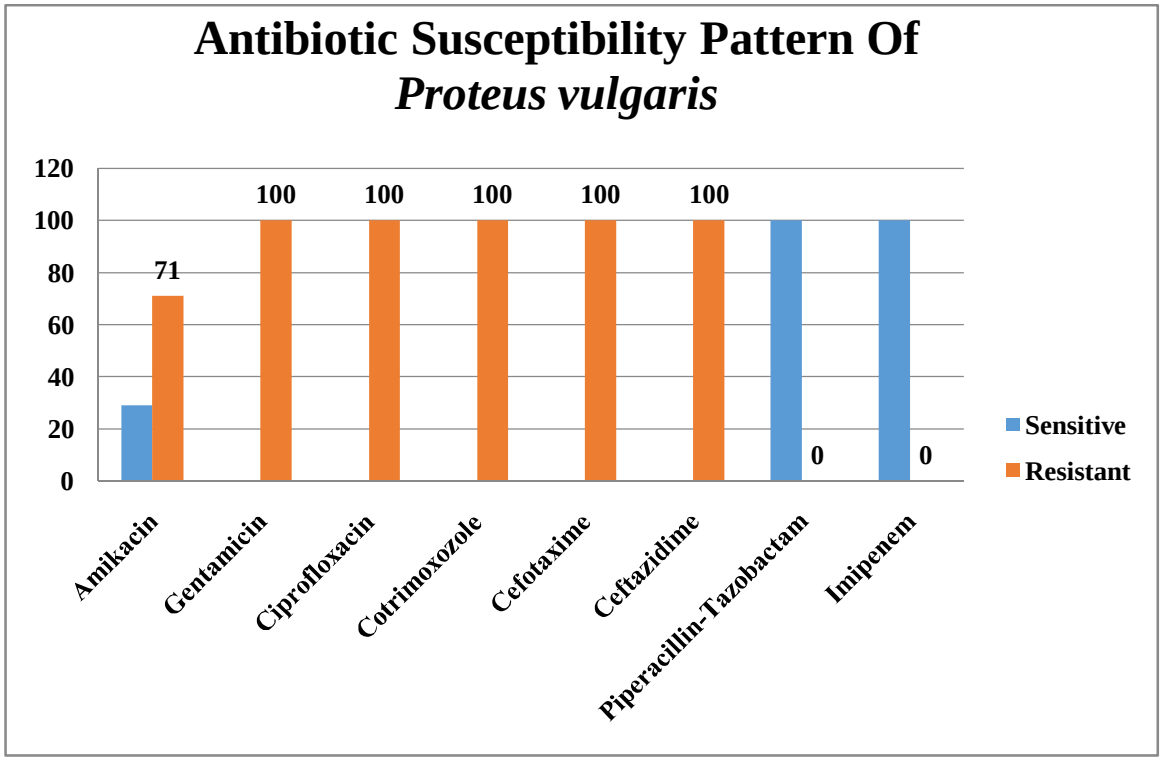


Antibiotic Susceptibility Pattern of *Proteus mirabilis*: (n=17)

Table:29

Antibiotic Susceptibility - *Proteus mirabilis* (n=17)	**Sensitive (%)**	**Resistant (%)**
Amikacin	53	47
Gentamicin	12	88
Ciprofloxacin	12	88
Cefotaxime	6	94
Ceftazidime	6	94
Cotrimoxozole	18	82
Imipenem	100	0
Piperacillin-Tazobactam	100	0

Figure 20

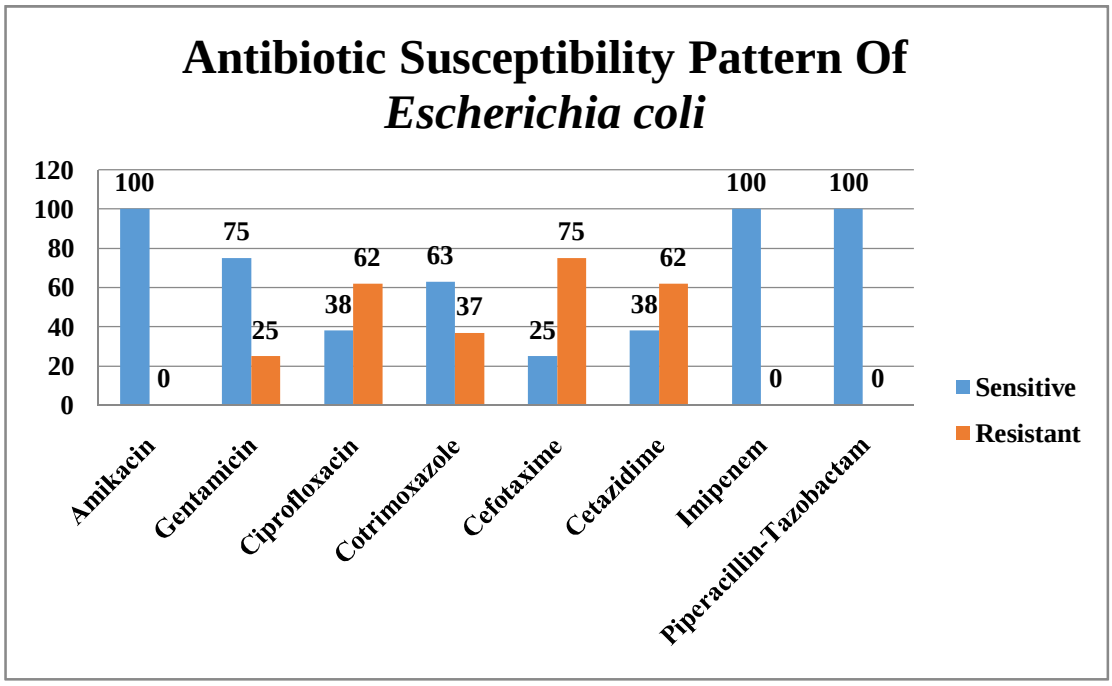


Antibiotic Susceptibility Pattern of *Proteus vulgaris*: (n=7)

Table:30

Antibiotic Susceptibility - *Proteus vulgaris* (n=7)	**Sensitive (%)**	**Resistant (%)**
Amikacin	29	71
Gentamicin	0	100
Ciprofloxacin	0	100
Cefotaxime	0	100
Ceftazidime	0	100
Cotrimoxozole	0	100
Imipenem	100	0
Piperacillin-Tazobactam	100	0

Figure 21

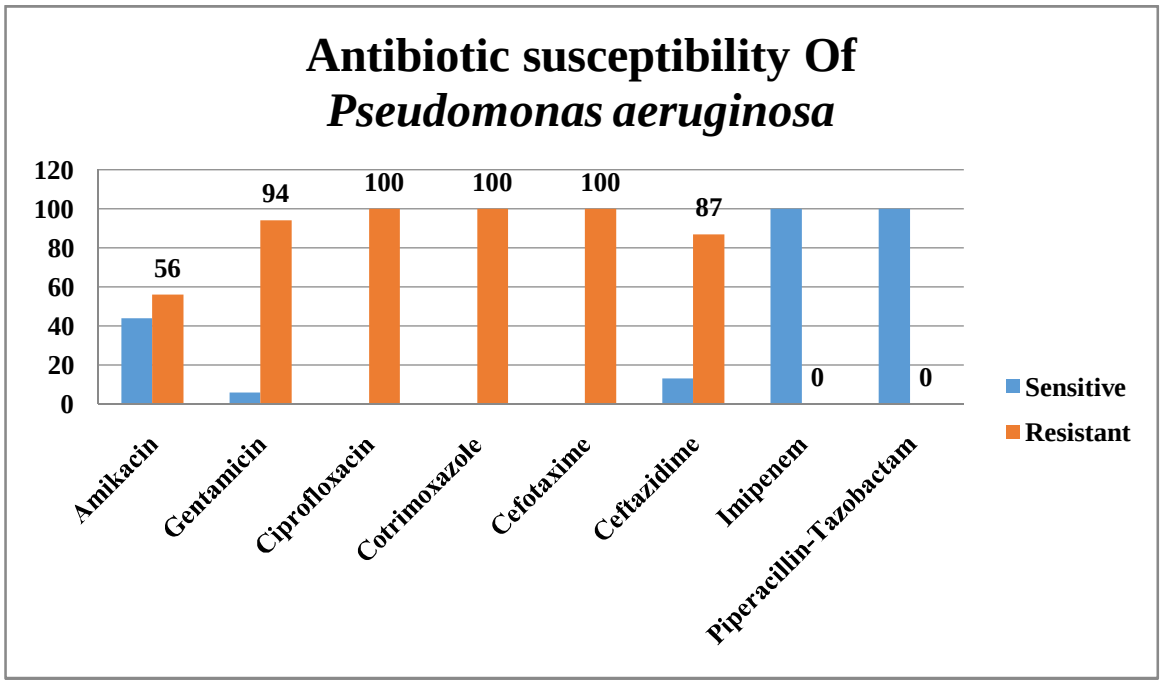


Antibiotic Susceptibility Pattern of *Escherichia coli*: (n=8)

Table:31

Antibiotic Susceptibility - *Escherichia coli* (n=8)	**Sensitive (%)**	**Resistant (%)**
Amikacin	100	0
Gentamicin	75	25
Ciprofloxacin	38	62
Cefotaxime	25	75
Ceftazidime	38	62
Cotrimoxozole	63	37
Imipenem	100	0
Piperacillin-Tazobactam	100	0

Figure 22

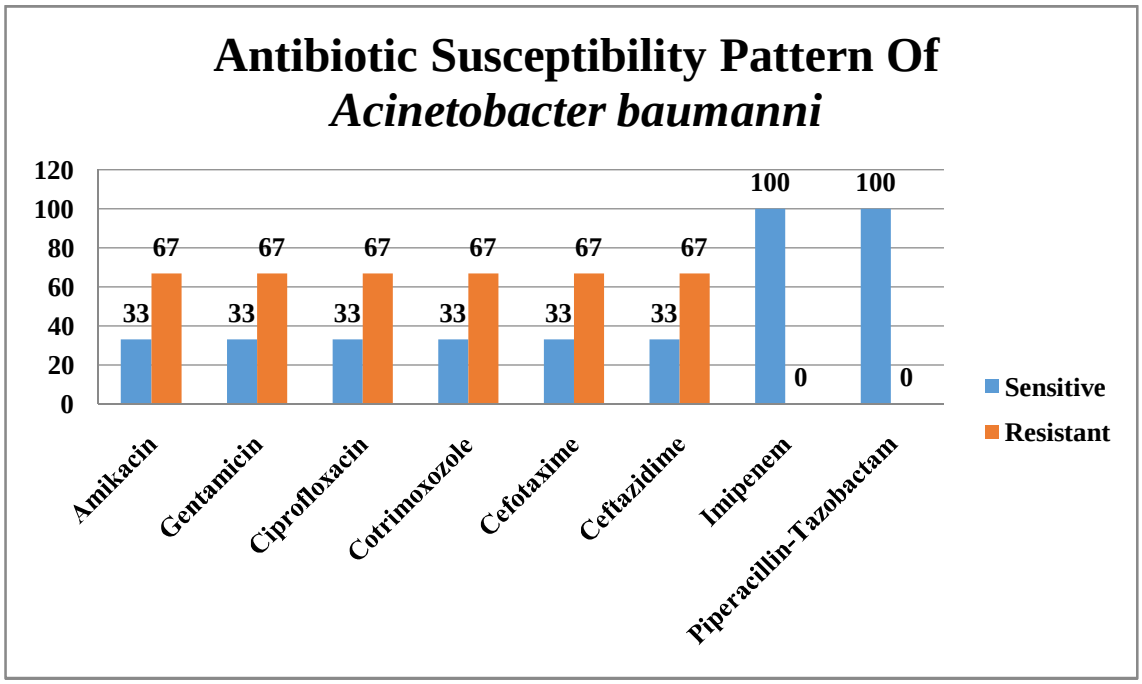


Antibiotic susceptibility of *Pseudomonas aeruginosa*: (n=16)

Table:32

Antibiotic Susceptibility - *Pseudomonas aeruginosa* (n=16)	**Sensitive (%)**	**Resistant (%)**
Amikacin	44	56
Gentamicin	6	94
Ciprofloxacin	0	100
Cefotaxime	0	100
Ceftazidime	13	87
Cotrimoxozole	0	100
Imipenem	100	0
Piperacillin-Tazobactam	100	0

Figure 23

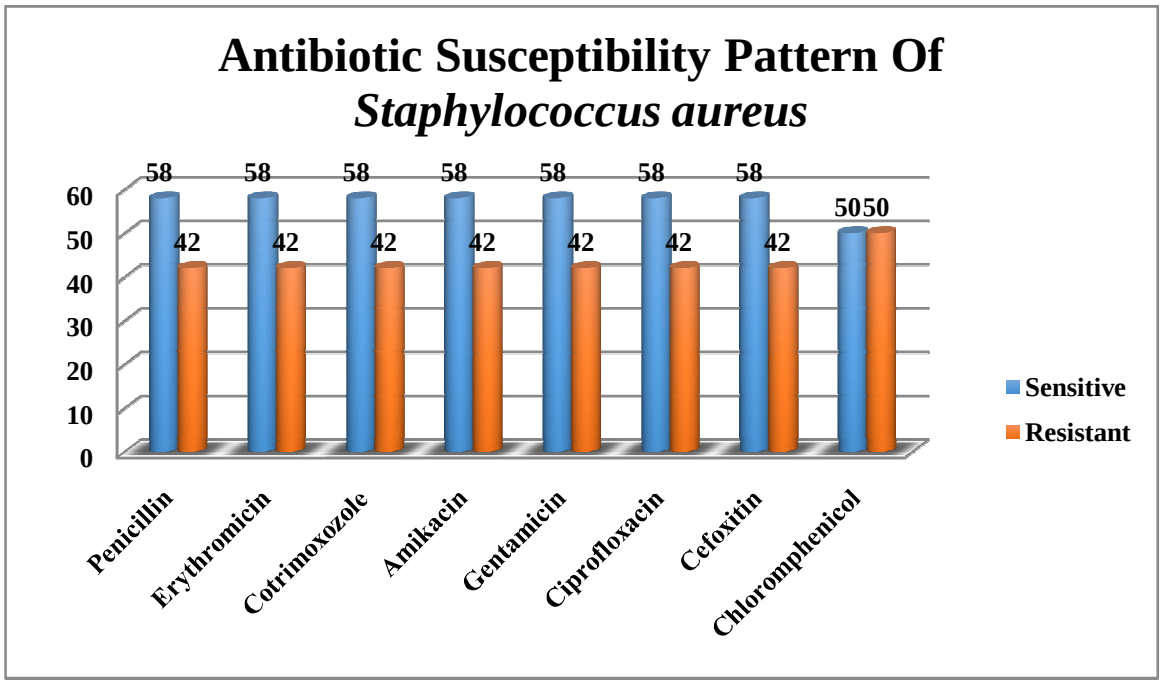


Antibiotic Susceptibility Pattern of *Acinetobacter baumanni*: (n=3)

Table:32

Antibiotic Susceptibility - *Acinetobacter baumanni* (n=3)	**Sensitive (%)**	**Resistant (%)**
Amikacin	33	67
Gentamicin	33	67
Ciprofloxacin	33	67
Cefotaxime	33	67
Ceftazidime	33	67
Cotrimoxozole	33	67
Imipenem	100	0
Piperacillin-Tazobactam	100	0

Figure 24

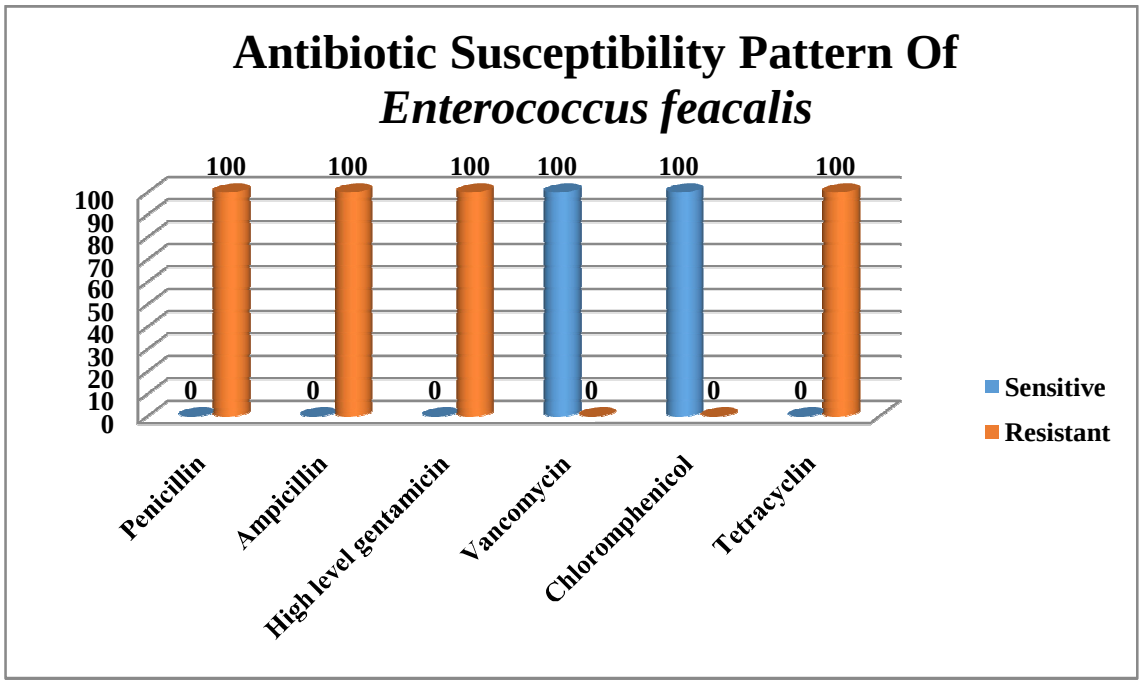


Antibiotic Susceptibility Pattern of *Staphylococcus aureus*: (n=12)

Table:33

Antibiotic Susceptibility - *Staphylococcus aureus* (n=12)	**Sensitive (%)**	**Resistant (%)**
Penicillin	58	42
Erythromycin	58	42
Cotrimoxozole	58	42
Amikacin	58	42
Gentamicin	58	42
Ciprofloxacin	58	42
Cefoxitin	58	42
Chloromphenicol	50	50

Figure 25



Antibiotic Susceptibility Pattern of *Enterococcus feacalis*: (n=4)

Table:34

Antibiotic Susceptibility - *Enterococcus feacalis* (n=4)	**Sensitive (%)**	**Resistant (%)**
Penicillin	0	100
Ampicillin	0	100
High level gentamicin	0	100
Vancomycin	100	0
Chloromphenicol	100	0
Tetracycline	0	100

Antibiotic susceptibility Pattern Of Aerobic Gram Negative Isolates: (n=94)

Table:35

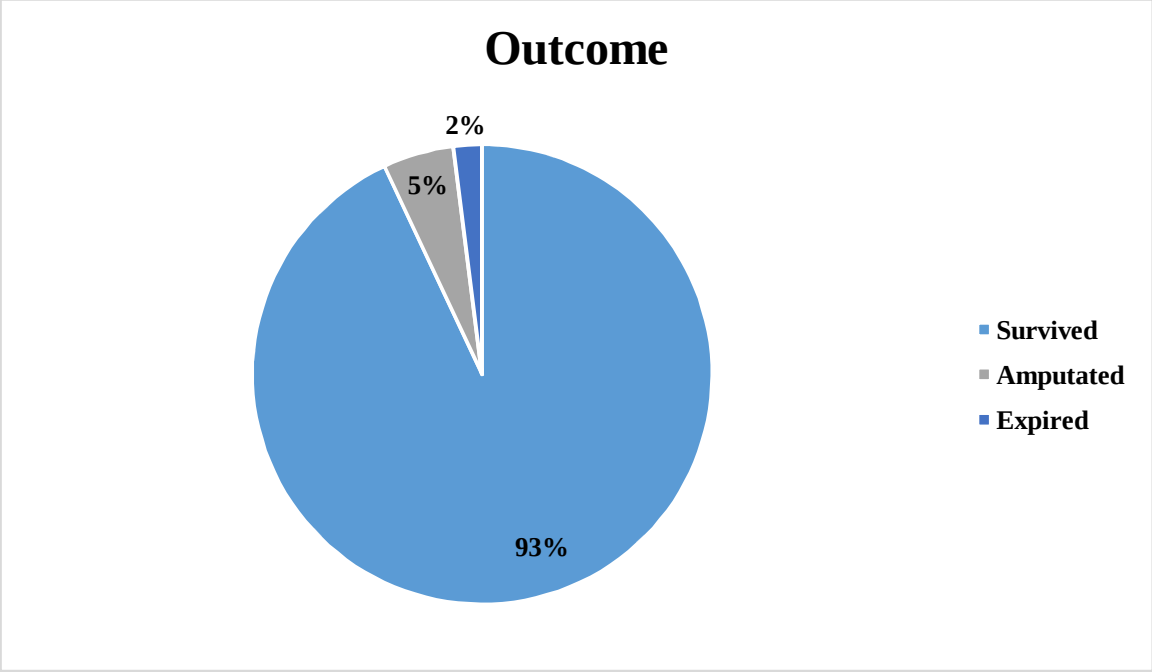
Antibiotic Susceptebility of Aerobic Gram negative bacilli (n=94)	*Klebsiella pneumoniae* (n=32)		*Klebsiella oxytoca* (n=11)		*Proteus mirabilis* (n=17)		*Proteus vulgaris* (n=7)		*Escherichia coli* (n=8)		*Pseudomonas aeruginosa* (n=16)		*Acinetobacter baumanni* (n=3)	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Amikacin	56	44	64	36	53	47	29	71	100	0	44	56	33	67
Gentamicin	44	56	27	73	12	88	0	100	75	25	6	94	33	67
Ciprofloxacin	38	62	18	82	12	88	0	100	38	62	0	100	33	67
Cefotaxime	28	72	0	100	6	94	0	100	25	75	0	100	33	67
Ceftazidime	28	72	0	100	6	94	0	100	38	62	13	87	33	67
Cotrimoxozole	50	50	27	73	18	82	o	100	63	37	0	100	33	67
Imipenem	100	0	100	0	100	0	100	0	100	0	100	0	100	0
Piperacillin-Tazobactam	100	0	100	0	100	0	100	0	100	0	100	0	100	0

Antibiotic susceptibility Pattern Of Aerobic Gram Positive Isolates: (n=16))

Table:36

Antibiotic Susceptebility of Aerobic Gram Positive Cocci (n=16)	***Staphylococcus aureus* (n=12)**		***Enterococcus feacalis* (n=4)**	
	Sensitive (%)	**Resistant (%)**	**Sensitive (%)**	**Resistant (%)**
Penicillin	58	42	0	100
Ampicillin	-	-	0	100
Erythromycin	58	42	-	-
Cotrimoxozole	58	42	\-	-
Amikacin	58	42	-	-
Gentamicin	58	42	-	-
High Level Gentamicin	-	-	0	100
Ciprofloxacin	58	42	-	-
Cefoxitin	58	42	-	-
Chloromphenicol	50	50	100	0
Vancomycin	-	-	100	0

Figure 26



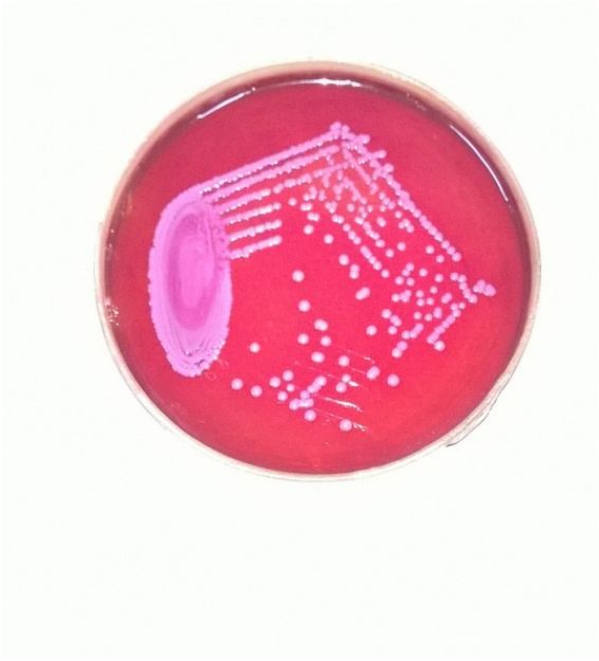
OUTCOME: (n=100)

Table:37

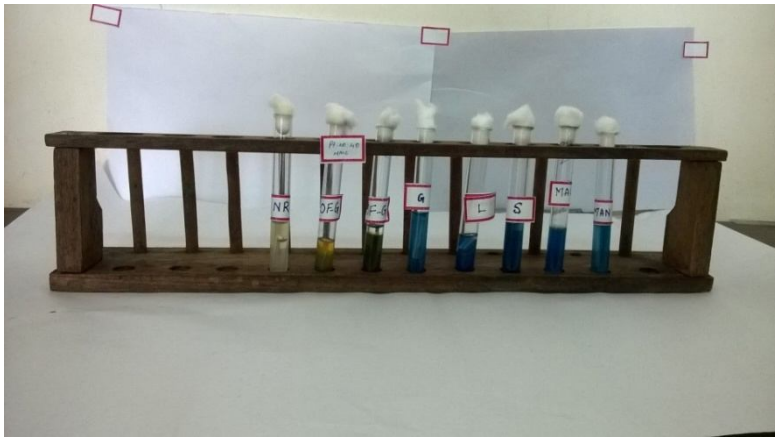
Outcome	Frequency	Percentage
Improved	93	93%
Amputated	5	5%
Expired	2	2%

NECROTISING FASCIITIS OF LEFT LOWER LIMB

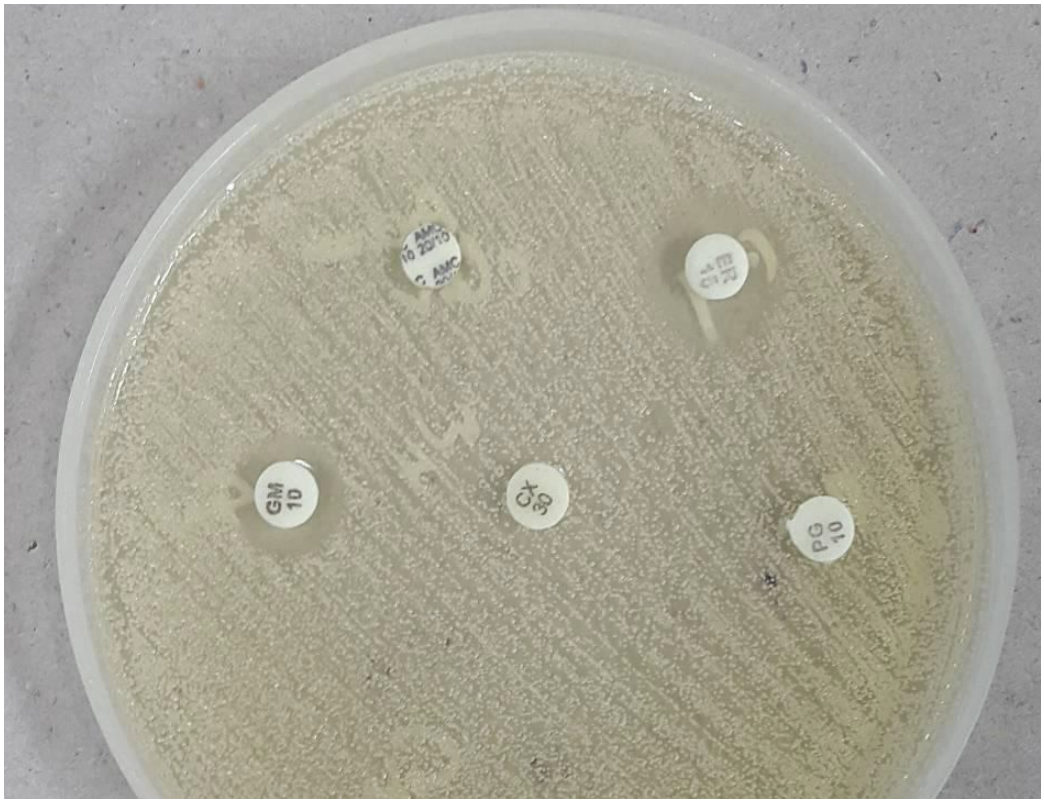
Klebsiella pneumonia



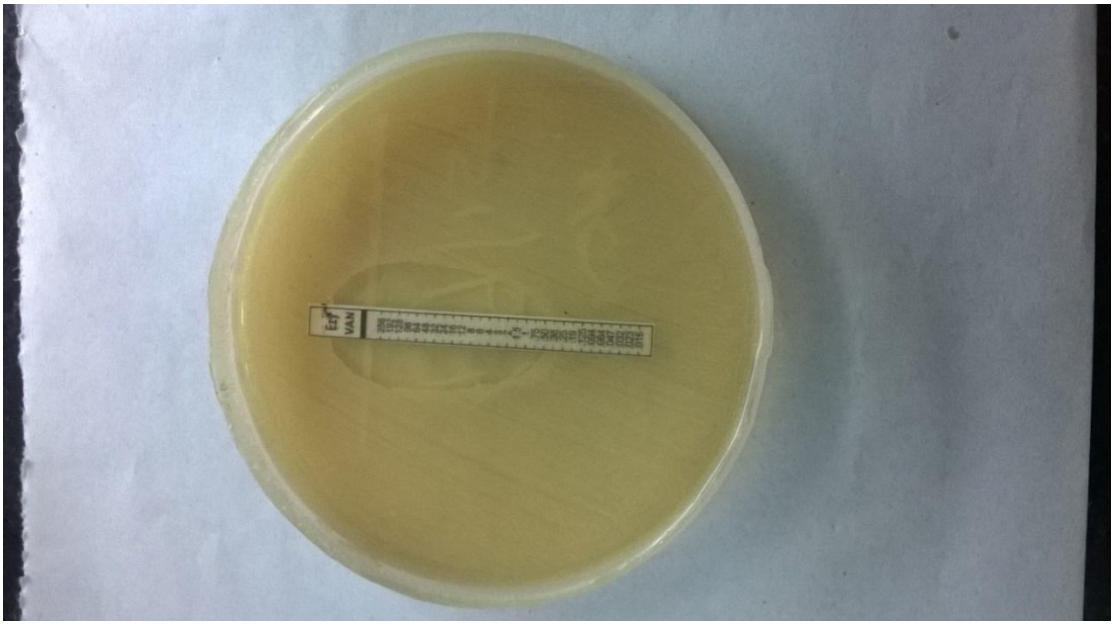
BIOCHEMICAL REACTIONS OF Klebsiella pneumonia



MRSA DETECTION BY CEFOXTIN DISC METHOD

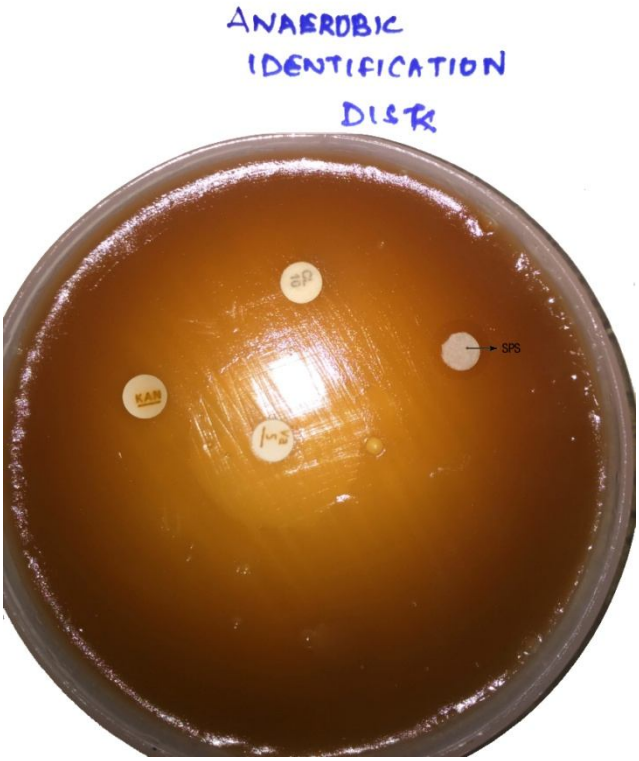


VANCOMYCIN MIC FOR MRSA BY E-TEST

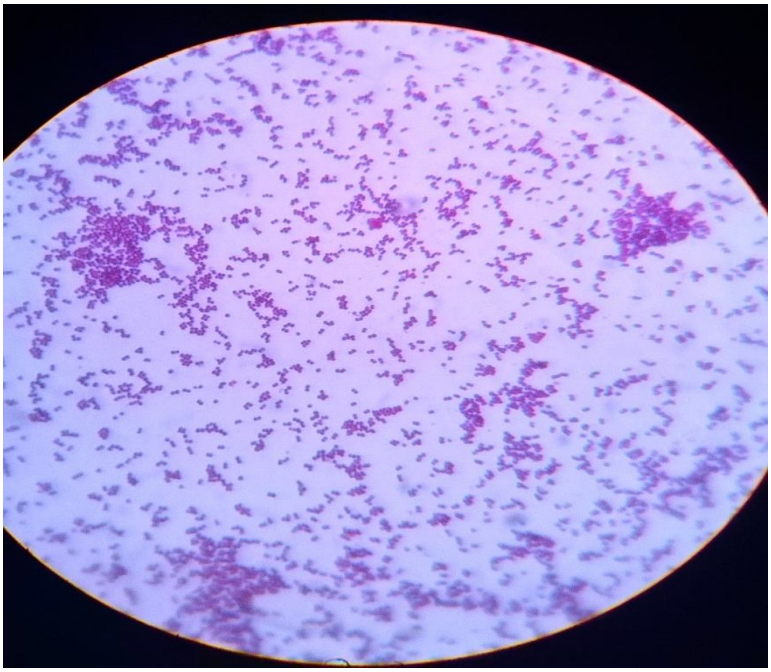


PHENOTYTIC ESBL CONFIRMATORY TEST

Peptostreptococcus anaerobius



Gram's stain shows Peptostreptococcus



DISCUSSION

This is a cross sectional study conducted in the Institute of Microbiology in association with the Institute of General surgery at Madras Medical College, Chennai. Duration of the study was one year and study population was one hundred patients of Necrotising Fasciitis.

Necrotising fasciitis was found to be more common in males than the female population. Out of the one hundred patients included in this study of the population males (n=76), 76% were more commonly affected by Necrotising Fasciitis. Females constituted (n=24), 24% of the study population. The peak incidence of NF was observed in patients aged between 51-60 years, which accounted for 24% of the study population. The mean age group was 58.35 years.

In this study extremities were more frequently affected sites, which was around 85%, of which lower limb happened to be the most common site which was involved in 83% of the patients. Scrotum (Fournier's gangrene) was affected in 11% of the patients. Increased frequency of lower limb infection corresponds to the fact that most of the patients were diabetics who were predisposed to lower limb infections. High blood sugar levels and low oxygen tension favours bacterial growth.

Tang et.al in their study series of 24 patients with necrotizing fasciitis of the limbs, had documented lower limb involvement in 12 patients. (53). Wong et.al in their study series of 89 patients, estimated that 70% of them had lower

limbs NF. (54) The preponderance of lower limb infection in the present study correlates with these studies.

In the present study, Type II or monomicrobial aetiology were found to be higher constituting 69% of infections and Type I, polymicrobial infections corresponded to 26% of infections. No growth was observed in specimens collected from five (5%) of the patients.

Some of the studies had reported significant rise in the incidence of monomicrobial infections. Ming- Jong Bair et al in a study had described that two third of infections were of monomicrobial aetiology. [55]

Yaug-Meng Liu et al has also showed 67.8% of monomicrobial agents as primary cause of Necrotising Fasciitis. [56]. D. Yadhav et al had retrospectively studied 45 patients with necrotising fasciitis documented to be of monomicrobial aetiology. [57]

Yao-Hung Tsai et al in a retrospective study had reported higher incidence (70.6%) of monomicrobial infection. [58]. The documentation of higher number of monomicrobial aetiological agent in this study is comparable with the other Asian studies.

Among the 69% of patients with monomicrobial infections, Gram negative bacilli were predominantly isolated in these patients. Enterobacteriaceae family members were the common isolates which constituted around 71%. Gram positive cocciisolated were *Staphylococcus aureus,* 10.9% and *Enterococcus feacalis,* 3.63%. Non- fermenters like

Acinetobacter baumanni and *Pseudomonas aeruginosa*, also accounted for 2.8% and 17.3% respectively as monomicrobial agents causing NF. *Klebsiella pneumoniae* was the common isolate constituting 26% of monomicrobial infections.

P- value obtained for monomicrobial growth by one sample Z test was 0.0414. Hence this is taken as statistically significant as this is lower than the significant value (0.05).

Patients with polymicrobial aetiology also had Enterobactriaceae members as the common pathogen with frequency of 66%. *Klebsiella pneumonia* showed a frequency of 33.3% isolate. *Staphylococcus aureus* 19.05% was isolated in patients with polymicrobial infections.

Yuang-Meng Liu et al in their study has depicted that Enterobactriaceae were the frequent pathogens, with *K. pneumoniae*being the most common organism isolated. (56). Though type I polymicrobial necrotising fasciitis has been the cause of 80% of cases as shown by several of the studies, there has been a substantial increase in incidence of monomicrobial necrotising fasciitis evident from recent studies. [59, 60].

In this study *Klebsiella pneumoniae* was the common organism isolated constituting of about 29.09% of the total isolates, followed by 15.45% of *Proteus mirabilis,* 14.54% of *Pseudomonas aeruginosa,*and 10.9% of *Staphylococcus aureus.*

*Enterococcus feacalis*3.63%, *Klebsiella oxytoca*10%, Proteus vulgaris 6.36%, *Acinetobacter baumanni* 2.72%, were the other pathogens which were relatively less frequent in the present study.

Klebsiella pneumonia, E.coli, Vibrio vulnificus, Aeromonas hydrophila were reported to be the most common isolates in Asia. [61, 62]. Fazal et al in their case series had reported 50.3% of *Klebsiella spp* infections. [2].

Mixed aerobic and anaerobic infection constituted about 50% of the polymicrobial infections. Mathew et al in their study had isolated 11.1% of mixed aerobic and anaerobic bacteria. [63]. Kreig et al retrospectively analysed patients with necrotising fasciitis between the year 1996 and 2005 and had reported that the cultures of 30.8% of the patients with type I polymicrobial infection also had anaerobes as synergistic pathogens. [64]

Of the one hundred patients, 13% had anaerobic infections. 61.5% was due to *Bacteroides fragilis* and 38.4% had *Peptostreptococcus anaerobius* infection. The virulence factors of these anaerobes had been documented in clinical infections. [65, 66]. Several hypothesis had been proposed to substantiate such microbial synergy. [67].

The synergy of bacteria may be explained by the following mechanisms;

(a)Due to mutual protection from phagocytosis and intracellular killing, [68]

(b) Production of essential growth factors, (69)

(c) Reduction of oxidation-reduction potential in host tissues, [70].

Type II diabetes was found to be the most frequent co-morbid condition associated with necrotising fasciitis accounting for 59% of the study population. Patients with both diabetes and hypertension constituted to 20%. One patient had type II diabetes mellitus with acute kidney injury. Type II DM with chronic Kidney disease was found in one patient. Out of the total one hundred patients, 4% of the patients had trauma as the main predisposing factor for NF and 10% had hypertension alone as co-morbidity. 5% of the study population presented with idiopathic necrotising fasciitis.

Several of the studies had analysed that Type II DM as the most common comorbid condition. Madhumita et al in a prospective study had reported Type II diabetes as the commonest co-morbidity. [71]. Yeung et al in their retrospective study had documented Type II DM as the frequent comorbidity. [72].

The above quoted studies had documented results that are comparable to this study. Type II DM has been documented as co-morbidity in 59%, (n=59%) of study population. P- Value calculated using one sample Z test was 0.0001, hence the association of type II DM and NF is statistically significant.

Trauma had been documented as a predisposing factor in 36.25% of the patients by Mathew et al. [63]. Nissar shaikh in his retrospective study had documented trauma as a predisposing Condition in 10% of the patients. [73]. 4% of patients had trauma as predisposing factor in this study.

Garg et al in a prospective study had reported several patients of necrotising fasciitis with no predisposing factors (Idiopathic NF). [74]. In the present study 5%of the patients presented with idiopathic necrotising fasciitis.

Klebsiella pneunomiae (n=32) were 56% sensitive to Amikacin, 44% sensitive to Gentamicin, 38% sensitive to ciprofloxacin, 50% sensitive to Co-trimoxozole and 28% sensitive to both cefotaxime and ceftazidime.

Out of the 12 isolates of *Staphylococcus aureus* 58% were sensitive to Penicillin, Erythromicin, Ciprofloxacin, Amikacin, Gentamicin, Co-trimoxozole. 50% were sensitive to Chloromphenicol.

Out of the 32 isolates of *Klebsiella pneumoniae*, 22 (40.74%) were Extended Spectrum Beta-Lactamase (ESBL) producers. 17 *Proteus mirabilis* were isolated, out of which 16 (29.63%) were ESBL producers. 11 *Klebsiella oxytoca* were isolated and 10 (18.52%) were found to produce ESBL. Of the 8 isolates of *Escherichia coli* 6 (11.11%) were ESBL producers.

By one sample Z test P-value was found to be 0.310 (lower than significant value 0.05) is considered to be statistically significant.

Anaya et al had had reported that 21.7% isolates of *Staphylococcus aureus* in patients of necrotising fasciitis. [1]. Angoules et al in their study had reported the presence of *Staphylococcus aureus* in 18.9% of the patients with necrotising fasciitis. [75]. The results of these studies corresponds to the results obtained in the present study. P-value obtained was 0.0169 by one sample Z test. This is taken as statistically significant.

Out of the 12 Staphylococcus aureus isolates, 5 (41.6%) were MRSA and 7 (58.3%) were MSSA. Chaun et al in a retrospective study had analysed and reported predominance of MSSA and MRSA isolates. [76]. Miller et al, in a retrospective study conducted in Los Angeles, had described 14 cases of Methicillin Resistant *Staphylococcus aureus*found to produce Panton valentine Leukocidin toxin, as the etiological agent of Necrotising fasciitis. [77]. Maltezou et al had documented MRSA causing necrotising fasciitis. [78].

Two patients (2%) with *Klebsiella pnemoniae* infection expired, out of one hundred patients included in the study. High mortality rate which was around 60% with *Klebsiella pneumonia* has been documented in a retrospective study by Yao-Hung Tsai. [58].

Several of the patients serially underwent two to three debridements during their hospital stay. Duration of hospitalisation of patients happened to be around thirty to forty five days in average. Five of the patients (5.1%) underwent below knee amputation. Out of the five patients four were diabetics and one other patient was a non- diabetic. Other patients improved during the course of their hospital stay.

Ramin et al in their study had reported significantly higher amputation (71.4%) in diabetics than in non-diabetics. [79].

All the patients received intravenous fluids and broad spectrum intravenous antibiotics. The antibiotics administered, included Piperacillin-Tazobactam, Metronidazole, Imipenem. Clindamycin, Imipenem and

combination of Penicillin and β-lactamase inhibitor target against anaerobic organisms. Imipenem, Piperacillin-Tazobactam combination also provides coverage for Enterobacteriaceae family members, and *Staphylococcus aureus*.

In the present study, *Klebsiella pneumoniae, Klebsiella oxytoca,Proteus mirabilis, Proreus vulgaris, Escherichia coli,Pseudomonas aeruginosa,Acinetobacter baumanni* were all 100% sensitive to Imipenem and Piperacillin-Tazobactam.

MRSA isolates were all sensitive to Vancomycin by Minimal inhibitory Concenteration by E-test.

SUMMARY

- This cross-sectional study was conducted at the Institute of Microbiology in association with the Institute of General Surgery, which included a study population of one hundred patients with Necrotising Fasciitis.
- Necrotising Fasciitis was common in males than in females included in the study population between the age group of 51-60 years.
- Lower limb was the frequent site of infection involved in majority of the patients and type II diabetes mellitus was the predominant co-morbid condition associated with necrotising fasciitis.
- Ninety five percentage of the growth obtained were aerobes and thirteen percentage were mixed aerobic and anaerobic infections.
- Enterobacteriaceae were the common isolates, of which *Klebsiella pneumoniae* was the most common pathogen isolated followed by Proteus mirabilis.
- *Bacteroides fragilis* was the predominant anaerobe isolated.
- *Klebsiella pneumoniae* was the predominant ESBL producer followed by *Proteus mirabilis.*
- Forty one percentage of *Staphylococcus aureus* were found to be Methicillin Resistant.
- All the Gram negative bacilli were sensitive to Imipenem and Piperacillin-Tazobactam.
- MRSA isolates were all sensitive to Vancomycin by E-test.

CONCLUSION

Necrotising Fasciitis is a rapidly progressing lethal condition where high degree of clinical suspicion, prompt diagnosis and intensive surgical and medical therapy by health care professionals should be the ultimate agenda to reduce morbidity and mortality. This study has documented the recent changes in the trend and frequency of bacteria causing necrotising fasciitis, which would be of immense assistance in selection and administration of appropriate empirical antibiotic therapy before the availability of culture reports.

Since there has been a remarkable surge in Gram negative Enterobacteriaceae members causing Necrotising Fasciitis, recognition of their antibiotic susceptibility pattern would offer a productive insight in affording potentially better medical therapy, in addition to the extensive surgical therapy. This would also aid in enhancement of recovery of the patients and thus limiting their duration of hospital confinement.

APPENDIX – I

ABBREVIATIONS

NF	–	Necrotising Fasciitis
NSTI	–	Skin and soft-tissue infection
PBSG	–	Progressive Bacterial Synergistic Gangrene
MODS	–	Multi- Organ Dysfunction Syndrome
CLSI	–	Clinical & Laboratory Standards Institute
ATCC	-–	American Type Culture Collections
MIC	–	Minimum Inhibitory Concentration
MRSA	–	Methicillin Resistant Staphylococcus aureus
MSSA	–	Methicillin Sensitive staphylococcus aureus
PVL	–	Panton Valentine leukocidin
ESBL	–	Extended Spectrum Beta Lactamase
SPS	–	Sodium Polyanetholsulfate

APPENDIX II

(1) Cation adjusted Mueller- Hinton Agar

Beef infusion 300ml

Caesein hydrolysate 17.5g

Starch 1.5g

Agar l0g

Distilled water litre

pH = 7.4

Sterilise by autoclaving at 121°C for 20 mins

(2) Robertson's Cooked Meat Broth

Fresh bullock heart 5 00g

Water 500ml

Sodium hydroxide, lmol/1 1.5ml

Liquid filtered from cooked meat 500ml

Peptone 2.5g

NaCl 1.25g

(3) Selective Anaerobic Blood Agar:

1 μg/ml menadione and 20 μg/ml gentamicin added to the blood agar.

ANNEXURE – I

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr.G.K.Asha
Postgradaute M.D.(Microbiology)
Madras Medical College
Chennai 600 003

Dear Dr.G.K.Asha,

The Institutional Ethics Committee has considered your request and approved your study titled **"A study on Bacteriological profile of Necrotising Fasciitis in a tertiary care hospital" No.21102014.**

The following members of Ethics Committee were present in the meeting held on 07.10.2014 conducted at Madras Medical College, Chennai-3.

1. Prof.C.Rajendran, M.D.,	: Chairperson
2. Prof.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
3. Prof.B.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
4. Prof.R.Nandhini, M.D., Inst.of Pharmacology, MMC	: Member
5. Prof.K.Ramadevi, Director i/c, Inst.of Bio-chem MMC	: Member
6. Prof.Saraswathy, M.D., Director, Pathology, MMC	: Member
7. Prof.S.G.Sivachidambaram, M.D., Director i/c, Inst. Of Internal Medicine, MMC	: Member
8. Thiru S.Rameshkumar,	: Lay Person
9. Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
10. Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

ANNEXURE – II

PROFORMA

Name: IP No:_____

Age:Ward:_____

Sex: M / F

Occupation:

Address:_____

_

_____

_

Presenting complaints:

Ulcers associated with pain and discharge

General examination:

Febrile/Afebrile

Icterus: Present/absent

Local examination:

Ulcers with erythema, oedema, blisters

Nature of discharge-purulent/watery

Presence of necrotic tissue

Depth of the ulcer

Provisional diagnosis:

Laboratory evaluation

Microbiological investigation:

Samples collected:

Swab from the deeper areas of the ulcers (OR)

Tissues obtained during wound debridement.

Direct examination

Gram’s Stain:

Aerobic and anaerobic culture:

Mac Conkey agar plate

Blood agar plate

Identification of Isolates

Antibacterial susceptibility pattern

ANNEXURE – III

CONSENT FORM

STUDY TITLE: A study on Bacteriological Profile of Necrotising Fasciitis in a tertiary care hospital."

I……, hereby give consent to participate in the study conducted by Dr.G.k.Asha, Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of analysis and to study the nature of the disease, I also give consent to give my sample for further investigations. I also learn that there is no additional risk in this study. I also give my consent for my investigator to publish the data in any forum or journal.

Signature/ Thumb impression of the patient/ relative

Patient Name & Address:

Place:

Date:

Signature of the investigator Signature of the guide

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MASTER CHART

PT NO	AGE	SEX	COMORBIDITY	SITE OF INFECTION	Organisms Isolated	AK	GEN	CIP	CEF	CAZ	PEN	AMPI	ERY	COTRI	CEFOXITIN	HLG	VAN(MIC)	VAN	IMI	PT	CK	TETRA	ESBL	MRSA	Anaerobes isolated
1	55	M	DM	NF Lt Lower limb	Klebsiella pneumoniae	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
2	56	M	DM, HT	NF Lt Lower limb	Pseudomonas aeruginosa	R	R	R	R	S	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
					Klebsiella pneumoniae	R	R	R	R	S	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
3	50	M	DM,HT	NF Rt Lower limb	Klebsiella pneumoniae	R	R	R	R	R	_	_	_	R	_	_	_	_	S	R	_	_	+	_	
4	60	M	DM	NF Lt Lower limb	Klebsiella pneumoniae	S	R	R	R	R	_	_	_	S	_	_	_	_	S	S	_	_	+	_	
5	56	F	DM	NF Lt Lower limb	Klebsiella pneumoniae	S	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
6	53	F	DM	NF Lt Inguinal region	Klebsiella pneumoniae	S	S	S	R	R	_	_	_	S	_	_	_	_	S	S	_	_	+	_	Bacteroides fragilis
7	70	M	DM	NF Lt Lowerlimb- gas gangrene	Klebsiella pneumoniae	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
8	50	M	DM,CKD	NF Rt Foot	Klebsiella pneumoniae	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	Peptostreptococcus anaerobius
					Proteus mirabilis	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
9	59	M	DM	NF Rt Foot	Klebsiella pneumoniae	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
10	62	M	DM	NF Rt Foot	Klebsiella pneumoniae	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
					Staphylococcus aureus	S	S	S	_	_	S	_	S	S	S	_	_	_	_	_	S	_	_	_	
11	40	M	DM	NF Lt Lower limb	Staphylococcus aureus	S	R	R	_	_	R	_	R	R	R		S	_	_	_	R	_	_	+	
12	65	F	DM	NF Rt Lower limb	Klebsiella pneumoniae	S	R	R	R	R	_	_	_	S	_	_	_	_	S	S	_	_	+	_	Bacteroides fragilis
13	42	M	DM	NF Rt Lower limb	Klebsiella pneumoniae	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
14	55	M	DM,HT	NF Lt Lower limb	Enterococcus fecalis	_	_	_	_	_	R	R	_	_	_	R	_	S	_	_	S	R	_	_	
15	35	M	Trauma	NF Lt Lower limb	Klebsiella pneumoniae	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
					Pseudomonas aeruginosa	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
16	70	M	DM,HT	NF Rt Lower limb	Staphylococcus aureus	S	S	S	_	_	S	_	S	S	S	_	_	_	_	_	R	_	_	_	Bacteroides fragilis
17	75	M	DM,HT	NF Rt Lower limb	Proteus vulgaris	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
18	73	M	DM	NF Rt Lower limb	Klebsiella pneumoniae	S	S	S	R	R	_	_	_	S	_	_	_	_	S	S	_	_	+	_	
					Staphylococcus aureus	S	R	R	_	_	R	_	R	R	R	_	S	_	_	_	S	_	_	+	
19	35	F	HT	NF Rt thigh	Proteus mirabilis	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	Peptostreptococcus anaerobius
20	82	F	DM	NF Lt Lower limb	Enterococcus fecalis	_	_	_	_	_	_	R	R	_	_	R	_	S	_	_	S	R	_	_	
21	65	F	DM	NF Rt Lower limb	Enterococcus fecalis	_	_	_	_	_	_	R	R	_	_	R	_	S	_	_	S	R	_	_	
22	65	M	DM	NF Lt Lower limb	Klebsiella pneumoniae	S	S	S	S	S	_	_	_	S	_	_	_	_	_	_	_	_	_	_	
23	45	M	DM,HT	NF Rt Lowerlimb- gas gangrene	Klebsiella pneumoniae	S	S	S	S	S	_	_	_	S	_	_	_	_	_	_	_	_	_	_	
					Staphylococcus aureus	S	S	S	_	_	S	_	S	S	S	_	_	_	_	_	R	_	_	_	
24	58	M	DM,HT	NF Rt Lower limb	Proteus mirabilis	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
25	86	M	DM	NF Rt Lower limb	Proteus mirabilis	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
26	67	F	DM	NF Lt Lower limb	Proteus vulgaris	S	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
27	80	M	DM	NF Lt Lower limb	Klebsiella pneumoniae	S	S	S	S	S	_	_	_	S	_	_	_	_	_	_	_	_	_	_	
28	26	M	Trauma	NF Lt Lower limb	Proteus mirabilis	S	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
29	80	M	DM,HT	NF Lt Lower limb	Klebsiella pneumoniae	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
30	75	M	DM	NF Lt Lower limb	Pseudomonas aeruginosa	S	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
31	18	M	Trauma	NF Rt Lower limb-gas gangrene	Klebsiella pneumoniae	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
					Pseudomonas aeruginosa	S	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	

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32	50	F	DM	NF Rt thigh	Escherichia coli	S	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
33	54	M	DM	NF Rt Lower limb	Staphylococcus aureus	S	R	S	_	_	R	_	R	R	R	_	S	_	_	_	S	_	_	+	
					Proteus mirabilis	S	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
34	67	F	DM	NF Lt Lower limb	Klebsiella oxytoca	S	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
35	42	F	DM	NF Lt Lower limb	Pseudomonas aeruginosa	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
36	45	M	DM,HT	NF Lt Hand	Proteus vulgaris	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	Peptostreptococcus anaerobius
37	60	M	DM	NF Lt Lower limb	Klebsiella pneumoniae	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
38	83	F	DM	NF Lt Lower limb	Escherichia coli	S	S	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
39	80	F	DM	NF Lt foot	Escherichia coli	S	S	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
40	70	M	DM,HT	NF Lt Lower limb	Staphylococcus aureus	S	S	S	_	_	S	_	S	S	S	_	_	_	_	_	S	_	_	_	
41	65	F	DM	NF Lt Lower limb	Proteus vulgaris	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	Bacteroides fragilis
42	82	F	DM	NF Rt foot	Proteus vulgaris	S	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
43	75	M	DM	NF Rt Lower limb	Pseudomonas aeruginosa	S	R	R	R	R	_	_	_	R	_	_	_	_	_	_	_	_	_	_	
44	73	M	DM	NF Rt Lower limb	Staphylococcus aureus	S	R	R	_	_	R	_	R	R	R	_	S	_	_	_	R	_	_	+	Bacteroides fragilis
					Klebsiella pneumoniae	S	S	S	S	S	_	_	_	S	_	_	_	_	_	_	_	_	_	_	
45	74	M	DM	NF Lt Lower limb	Klebsiella pneumoniae	S	S	S	S	S	_	_	_	S	_	_	_	_	_	_	_	_	_	_	
					Staphylococcus aureus	S	S	S	_	_	S	_	S	S	S	_	_	_	_	_	R	_	_	_	
46	58	M	DM	NF Rt Lower limb	Proteus mirabilis	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
47	55	M	DM	NF Lt Lower limb	NO GROWTH																			_	
48	80	M	DM,HT	NF Lt Lower limb	Proteus mirabilis	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
49	55	F	DM,HT	Fournier's gangrene	Proteus mirabilis	S	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
50	26	M	idiopathic	NF Lt Lower limb	Pseudomonas aeruginosa	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
51	54	M	DM		Klebsiella pneumoniae	S	S	S	S	S	_	_	_	S	_	_	_	_	_	_	_	_	_	_	
52	80	M	HT	NF Rt Lower limb	Klebsiella pneumoniae	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
53	75	M	DM,HT	NF Lt Lower limb	Pseudomonas aeruginosa	S	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
54	18	M	Idiopathic	NF Rt foot	NO GROWTH																			_	
55	53	M	DM	NF Lt Lower limb	Pseudomonas aeruginosa	S	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
56	67	M	DM	NF Lt Lower limb	Klebsiella oxytoca	S	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
57	45	M	HT	NF Rt hand	Proteus vulgaris	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	Peptostreptococcus anaerobius
58	42	F	HT	NF Rt Lower limb	Pseudomonas aeruginosa	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
59	29	M	Idiopathic	Fournier's gangrene	Escherichia coli	S	S	S	S	S	_	_	_	S	_	_	_	_	_	_	_	_	_	_	
60	73	M	DM,HT	NF Lt Lower limb	Pseudomonas aeruginosa	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
61	80	M	DM	Fournier's gangrene	NO GROWTH																			_	
62	63	M	HT	NF Lt Lower limb	Proteus mirabilis	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
					Klebsiella oxytoca	R	R	R	R	R	_	_	_	_	_	_	_	_	S	S	_	_	+	_	
63	45	M	HT	NF Rt Lower limb	Eschericia coli	S	R	R	R	S	_	_	_	S	_	_	_	_	S	S	_	_	+	_	
64	73	M	HT	NF Rt Lower limb	Pseudomonas aeruginosa	R	R	R	R	S	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
65	25	M	Traumatic	NF Lt Lower limb	Klebsiella oxytoca	S	S	S	S	S	_	_	_	S	_	_	_	_	_	_	_	_	_	_	
66	66	M	DM	Fournier's gangrene	Acinetobacter baumanni	S	S	S	S	S	_	_	_	S	_	_	_	_	_	_	_	_	_	_	Peptostreptococcus anaerobius

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67	50	F	DM	NF Lt Lower limb	Pseudomonas aeruginosa	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
68	54	F	DM,HT	NF Lt Lower limb	Klebsiella pneumoniae	S	S	S	S	S	_	_	_	S	_	_	_	_	_	_	_	_	_	_	
69	62	F	DM,HT	NF Lt Lower limb	Proteus mirabilis	S	S	S	S	S	_	_	_	S	_	_	_	_	_	_	_	_	_	_	
70	40	M	DM	NF Lt Lower limb	Eschericia coli	S	S	S	S	S	_	_	_	S	_	_	_	_	_	_	_	_	_	_	
71	60	M	DM	Fournier's gangrene	Pseudomonas aeruginosa	S	S	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
72	50	M	DM	NF Lt Lower limb	Klebsiella oxytoca	S	R	R	R	R	_	_	_	S	_	_	_	_	S	S	_	_	+	_	
73	75	M	HT	NF Rt Lower limb	Eschericia coli	S	S	S	R	R	_	_	_	S	_	_	_	_	S	S	_	_	+	_	
74	78	M	DM	NF Rt foot	Proteus mirabilis	S	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
75	45	M	DM	NF Lt Lower limb	Klebsiella pneumoniae	S	S	S	S	S	_	_	_	S	_	_	_	_	_	_	_	_	_	_	
76	50	M	DM	Fournier's gangrene	Eschericia coli	S	S	R	R	R	_	_	_	S	_	_	_	_	S	S	_	_	+	_	
77	70	M	DM, AKI	NF Rt Lower limb	Klebsiella pneumoniae	S	R	R	R	R	_	_	_	S	_	_	_	_	S	S	_	_	+	_	
78	55	M	DM	NF Rt foot	Klebsiella oxytoca	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
					Proteus mirabilis	S	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
79	54	F	HT	NF Lt lower limb	NO GROWTH												_							_	
80	45	M	DM	NF Lt lower limb	Proteus mirabilis	S	S	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
					Klebsiella oxytoca	S	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
81	59	M	DM	NF Lt foot	Klebsiella pneumoniae	S	S	R	R	R	_	_	_	S	_	_	_	_	S	S	_	_	+	_	Bacteroides fragilis
82	50	M	DM,HT	NF Rt lower limb	Proteus mirabilis	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	Bacteroides fragilis
83	50	M	DM	NF Lt lowerlimb	Staphylococcus aureus	S	R	S	_	_	R	_	R	R	R	_	S	_	_	_	S	_	_	+	
84	50	M	DM	Fournier's gangrene	Klebsiella oxytoca	S	S	S	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
85	29	M	Idiopathic	Fournier's gangrene	NO GROWTH												_							_	
86	65	F	DM	NF Rt lower limb	Pseudomonas aeruginosa	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
87	74	F	DM	NF Rt lower limb	Klebsiella pneumoniae	S	S	S	R	R	_	_	_	S	_	_	_	_	S	S	_	_	+	_	
88	75	M	DM,HT	NF Lt lowerlimb	Klebsiella oxytoca	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
89	65	M	DM	NF Lt lowerlimb	Pseudomonas aeruginosa	S	R	R	R	R	_	_	_	R		_	_	_	S	S	_	_	_	_	
90	53	M	DM	NF Lt Lowerlimb	Staphylococcus aureus	S	S	S	_	_	S	_	S	S	S	_	_	_	_	_	S	_	_	_	
91	50	M	DM	Fournier's gangrene	Proteus vulgaris	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
92	54	M	DM,HT	NF Lt foot	Klebsiella pneumoniae	S	S	S	S	S	_	_	_	S	_	_	_	_	_	_	_	_	_	_	
93	78	M	DM	NF Lt lowerlimb	Acinetobacter baumanni	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S		_	_	_	
94	64	F	HT	NF Rt lower limb	Klebsiella pneumoniae	S	S	R	R	R	_	_	_	S	_	_	_	_	S	S	_	_	+	_	
95	27	F	Idiopathic	NF-Perianal	Enterococcus feacalis	_	_	_	_	_	R	R	_	_	_	R	_	S	_	_	S	R	_	_	
					Klebsiella oxytoca	S	S	R	R	R	_	_	_	S	_	_	_	_	S	S	_	_	+	_	
96	57	M	DM	NF Rt lower limb	Proteus mirabilis	S	R	R	R	R	_	_	_	S	_	_	_	_	S	S	_	_	+	_	
97	74	M	DM	Fournier's gangrene	Proteus mirabilis	S	R	S	R	R	_	_	_	S	_	_	_	_	S	S	_	_	+	_	
98	63	M	DM	NF Rt lower limb	Acinetobacter baumanni	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	_	_	
99	65	M	DM,HT	NF Lt lower limb	Klebsiella oxytoca	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	
100	55	M	DM	NF Rt lower limb-gas gangrene	Klebsiella pneumoniae	R	R	R	R	R	_	_	_	R	_	_	_	_	S	S	_	_	+	_	Bacteroides fragilis
					Staphylococcus aureus	S	S	S	_	_	S	_	S	S	S	_	_	_	_	_	R	_	_	_	

Keys to Master Chart

Ak	–	Amikacin
Gen	–	Gentamicin
Cip	–	Ciprofloxacin
Cotri	–	Cotrimoxozole
Cef	–	Cefotaxime
CAZ	–	Ceftazidime
Imi	–	Imipenem
PT	–	Piperacillin-Tazobactam
Pen	–	Penicillin
Ery	–	Erythromicin
HLG	–	High level gentamicin
Van	–	Vancomycin
Ck	–	Chloromphenicol
Tetra	–	Tetracyclin
S	–	Sensitive
R	–	Resistant
(-)	–	Not applicable